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Influence of Environmental Factors on the Seed Ecology of Vallisneria americana

Jessie J. Campbell
College of William and Mary - Virginia Institute of Marine Science

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE SEED ECOLOGY OF VALLISNERIA AMERICANA

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Jessie J. Campbell

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Kenneth A. Moore, Ph.D.
Committee Chairman, Advisor

Carl H. Hobbs, Ph.D.

Robert J. Orth, Ph.D.

James E. Perry, III, Ph.D.

Linda C. Schaffner, Ph.D.

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ABSTRACT

Environmental conditions may positively or negatively influence production, reproduction, and restoration of submerged aquatic vegetation (SAV). As SAV populations decline, it is important to understand the potential impacts of the environment on the natural processes of reproduction. The objectives of this study are to provide fundamental information on sexual reproduction in a dominant species of freshwater SAV, *Vallisneria americana,* in the Chesapeake Bay observe the changes in environmental conditions in an established *V. americana* bed over an entire growing season (April-October), quantify the effects of similar environmental conditions on *V. americana* seed germination under controlled laboratory conditions, and finally to synthesize this information in order to develop a series of criteria for *V. americana* restoration in the Chesapeake Bay.

Light, dissolved oxygen, temperature, salinity, sediment composition, *V. americana* production (biomass, density) and reproduction (flowering) were measured in a persistently vegetated SAV bed located in a tidal freshwater tributary to the Chesapeake Bay over the 2004 growing season. In addition, the effects of these same parameters on germination of *V. americana* seeds were quantified separately under controlled laboratory conditions. Production of *V. americana* biomass in Nanjemoy Creek increased significantly when water temperatures increased above a threshold of 25 °C. Flowering occurred during periods of peak biomass (August and September) and resulted in the production of up to 71 seed pods m^{-2} at the end of the growing season. Despite the potential production of thousands of seeds m^{-2}, < one percent were retained in the seed bank and remained viable after a period of 12 months. Germination of *V. americana* seeds was enhanced (greater overall germination and shorter time to germination) under oxygenated conditions, at temperatures > 22 °C, at salinities of 0 psu, and when sediments were composed of ≤ 3 percent organic content and > 40 percent sand. Light (< 160 μmol m^{-2} s^{-1}) and burial depth (down to 10 cm) had no significant effect on germination.

Based on the synthesis of field and laboratory data, criteria for restoration of *V. americana* using seeds in Chesapeake Bay should include temperatures greater than 13 °C, salinities ≤ 5 psu, and sediment containing ≤ 3 percent organic content and > 40 percent sand. This research provides the initial steps to determine which environmental factors affect germination of *V. americana* seeds and may increase the efficiency of current restoration practices for this species and provide a mechanism for increased success of current and potential larger scale projects.

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE SEED ECOLOGY OF *VALLISNERIA AMERICANA*

INTRODUCTION

Submerged aquatic vegetation (SAV) beds are important components of estuarine ecosystems. Both marine and freshwater rooted angiosperm communities provide habitat, protection, and nursery functions for economically valuable fishery species (Kilgore et al., 1989, Duffy and Baltz, 1998; Richardson et al., 1998), are primary sources of food for waterfowl (Perry and Uhler, 1988; Perry and Deller, 1996), serve as indicators of and modify local water quality conditions (Dennison et al., 1993; Moore, 2004), and decrease shoreline erosion by dampening nearshore waves (Fonseca and Cahalan, 1992). Studies of the Chesapeake Bay estuary have shown major fluctuations and overall declines in SAV populations since the 1960's (Bayley et al., 1968, 1978; Stevenson and Confer, 1978; Orth and Moore, 1983). Many of the Chesapeake's major tributaries support only sparse coverage of SAV where historical photographic evidence shows there were once extensive populations (Moore et al., 2004). Despite numerous restoration efforts, there has only been a slight increase in SAV populations over the last several years (Moore et al., 2000; Orth et al., 2004; Figure 1).

Numerous hypotheses accounting for the decline of SAV populations in coastal systems have been proposed over the last 30 years. Many address issues related to water quality conditions including decreased light availability to SAV in response to increased phytoplankton biomass (Phillips et al., 1978), decreased light availability resulting from increased periphyton accumulation on the blades of SAV (Sand-Jensen, 1977; Dennison et al., 1993; Neckles et al., 1993; Nelson and Waaland, 1997), and burial of SAV and decreased light availability due to increased sediment inputs (Bayley et al., 1978; Rybicki and Carter, 1986). Other factors implicated in SAV decline include introduction of and

resulting competition with non-native species (Bayley et al., 1978; Orth and Moore, 1984) and reproductive failure (Titus and Hoover, 1991). The potential impacts of many of these factors combined with limited restoration success on a global scale require an increased effort to understand how SAV become established, successfully survive, and reproduce in their current environments (Inglis, 2000).

Reproduction of SAV has been investigated for several decades. Submerged macrophytes are capable of both sexual and vegetative reproduction (Figure 2), with the majority of reproduction reported to occur vegetatively (Sculthrope, 1967; Hutchinson, 1975). Limited attention has been paid to sexual reproduction due to the prevalence of clonal individuals in most populations and the infrequent occurrence of seedlings (Stevenson, 1988; Barrett et al., 1993; Hemminga and Duarte, 2000). The balance between clonal organisms and seedlings is hypothetically based on a strategy of maximizing local populations while utilizing all suitable habitats within a specific region (Inglis 2000; Santamaria, 2002). There are two hypotheses that address the success of vegetative reproduction in SAV. First, most SAV do not produce the quantity of seeds and seedlings necessary to compete with vegetative propagation (Eriksson and Ehrlen, 1992), and second many habitats where seeds are deposited are not adequate for seedling establishment (Crawly, 1990). These processes occur concurrently and the lack of seedlings in a clonal population is often thought to be the result of several complex interactions (Eriksson and Ehrlen, 1992).

Seed production and seedling establishment do, however, serve important functions in communities of SAV. Seeds provide genetic variability within populations (Titus and Hoover, 1991; Waycott, 1995), compensate for seasonal die-off (McMillan

and Jewett-Smith, 1988; Titus and Hoover, 1991), increase disease resistance (Lokker et al., 1997), enhance recovery from large-scale storm or flooding events (Birch and Birch, 1984; Boins and Lepart, 1994; Preen et al., 1995), and provide a mechanism for increasing the geographic range and establishment of new communities (Arnold et al., 2000; Figuerola et al., 2002; Green et al., 2002; Harwell and Orth 2002). Despite the progress made into understanding the ecological functions of seeds in SAV communities, little is known about the factors that affect the germination and establishment of SAV seeds. The intent of this study is to investigate the basic relationships between environmental factors and seed germination for one dominant species of freshwater SAV in the Chesapeake Bay, *Vallisneria americana,* to help correct this lack of knowledge.

Ecology of *Vallisneria americana*

Morphological Description and Geographic Distribution

Vallisneria americana Michx.(wild celery), a member of the family Hydrocharitaceae, is a perennial, dioecious, freshwater angiosperm characterized by leaves that are finely toothed with a distinct mid-vein. Unlike most other freshwater SAV, which form a canopy on the surface of the water, *V. americana* leaves grow to lengths up to 2 meters long from a basal meristem and form meadows (Wilder, 1974; Catling et al., 1994). In North America *V. americana* is distributed along the Atlantic and Gulf of Mexico coasts and in interior areas from Nova Scotia to South Dakota (Lowden, 1982; Adair et al., 1994).

Habitat Requirements

The broad distribution of *V. americana* reflects its ability to live in a variety of environmental conditions (Bayley et al., 1978; Korschgen and Green, 1988). These conditions include sandy to soft clay substrates (Rybicki and Carter, 1986; Adair et al., 1994), moderate to low light environments receiving minimums of 5 – 25 percent total surface irradiance (Meyer et al., 1943; Carter and Rybicki, 1985; Doyle and Smart, 2001), median temperatures ranging from 19°C to 36°C (Barko et al., 1982; Kraemer et al., 1999), and variable salinity regimes with optimum conditions between 3 to 5 psu (Steenis, 1970; French and Moore, 2003) in both lentic and lotic systems (Korschgen and Green, 1988; Doyle, 2001).

Life Cycle and Reproduction

Vallisneria americana is a historically dominant freshwater species in Chesapeake Bay (Davis, 1985). The annual cycle of *V. americana* in Chesapeake Bay begins when new growth emerges from over-wintering buds or seeds in April. Growth (including the production of new leaves and leaf cluster or rosettes) occurs until late September with peak biomass evident in August. Flowering lasts from July through September. Turions (the results of vegetative propagation), seed pods, and winter buds are produced during September and October. Once the rosettes die back in November, the seeds and winter buds remain in the sediments until the next spring (Korschgen and Green, 1988; Catling et al., 1994).

Vegetative propagation by winter buds is the primary reproductive mechanism for *V. americana* (Sculthorpe, 1967; Lowden, 1982). In the early fall *V. americana* leaf

production stops and winter buds are produced by stolons. After winter-bud development, the stolons elongate until they are buried in the sediment. The remaining plant tissue dies off and separates from the substrate while the winter buds remain in the sediment until the following spring. One winter bud can produce between 20 to 40 new plants (Sculthorpe, 1967; Titus and Stephens, 1983).

Sexual reproduction of *V. americana* occurs during the months of July, August, and September in the Chesapeake Bay region (Korschgen and Green, 1988). Once pistillate flowers are produced, they elongate until reaching the water's surface. Staminate flowers break from their basal stems and float along the water's surface (Cox, 1993; Titus and Hoover, 1991). Pollination occurs when the staminate flower, propelled by either water currents or wind, dips into the depression created by the pistillate flower and pollen is deposited onto the stigma (Sculthrope, 1967; Kaul, 1978). The pistillate flower is pulled beneath the surface by the coiling of its stem where approximately 100-300 seeds contained in a gelatinous mass form within the resulting seed pod (Lokker et al., 1997). Seed pods are positively buoyant and once separated from the stem can either float away or rupture dispersing seeds in the same distinct community (Titus and Hoover, 1991; Lokker et al., 1997).

Studies on SAV seeds and seedlings

Ecosystem Function of Seeds

Seed banks are defined as areas containing viable seeds that are found on or in the sediment (DeBerry and Perry, 2000). Studies of wetland seed banks have shown these storage areas to be important in establishing and maintaining wetlands, re-establishing

populations after disturbances, and increasing biodiversity (van der Valk, 1981; Thompson, 1992). Despite studies with similar results concerning SAV, few have studied the seed bank for clonal species like *V. americana*. Lokker et al. (1997) observed that *V. americana* seeds form seed banks near established SAV beds in the Lake Huron-Erie corridor. The number of seeds stored in the seed bank was 10 times less than the number of seeds produced in the same area; however, seed predation, dispersal, as well as germination of seeds during the growing season could account for the difference. Similar results were found in Lake Ontario in SAV beds near marshes (Westcott et al., 1997).

For many SAV species, the primary function of seeds is not seed bank formation, but to provide a mechanism for long distance dispersal (Harwell and Orth, 2002). In the Chesapeake Bay seeds of *Zostera marina* L. (eelgrass) attached to reproductive shoots are capable of dispersing up to 100 km away from the parent bed (Harwell and Orth, 2002). In the freshwater environment seeds of the SAV species *Myriophyllum spicatum* L. (Eurasian milfoil) are hypothesized to be responsible for reestablishment of the species after disturbance and to serve as a long-term survival mechanism (Hartleb et al., 1993). Whether or not *V. americana* seeds serve as a long distance dispersal mechanism is unknown.

In Chesapeake Bay seeds and shoots of *V. americana* and other species of SAV are a major food source for waterfowl (Perry and Deller, 1996). Dispersal of freshwater SAV seeds via waterfowl through both internal (gut) and external (attachment to feathers) methods is the primary mechanism for establishment of new populations into these systems (Santamaria and Klaassen, 2002; Figuerola et al., 2003; Higgins et al., 2003). Seed dispersal through waterfowl consumption is not limited to one species or to the time

of seed production. For example, seeds of 15 plant species (including *Potamogeton pectinatus* L. (sago pondweed) and *Ruppia maritima* L. (widgeon grass)) were attached to the plumage of 6 species of waterfowl on 35 percent of individual birds sampled (47 individuals total) and seeds were dispersed through the fall and winter (Figuerola and Green, 2002; Figuerola et al., 2002). For these species of freshwater SAV, seeds were responsible for dispersal over long distances and subsequent establishment of new beds.

There is a lack of information concerning the function of seeds, ability of seeds to germinate, the ability of germinated seeds to become established, and recorded observations of established seedlings in the field (Sculthorpe, 1967). Titus and Hoover (1991) demonstrated that 67 percent of *V. americana* seedlings transplanted in to Oswego Lake, New York, became established and produced new rosettes within 4 months. In the upper Mississippi River, *V. americana* seedlings became established in ambient conditions, survived the entire growing season, and produced turions within one year of planting (Kimber et al., 1995). While experimental evidence of germination and establishment of *V. americana* seeds does provide information on the ability of seedling to become established, it does not explain the lack of observed seedlings in the field.

Seed Production and Germination

Environmental factors may influence *V. americana* germination rates and seedling establishment before seeds are even produced. Inadequate genetic information (Lokker et al., 1997); hydrophobic pollen (Cox, 1993); inefficiency of pollen dispersal in a three-dimensional habitat (Cox, 1993); and stress of parent plants due to environmental factors during seed production (Fussel and Pearson, 1980; Grass and Burris, 1995) are all

possible hypotheses for an observed lack of seedlings in natural SAV assemblages. Overall, environmental factors such as temperature and salinity are found to be the main influence on flowering, production, and viability of seeds in both aquatic and terrestrial plants.

Effects of Environmental Factors on Germination

Major influences of temperature, salinity, and, to a lesser degree, light, oxygen, and sediment composition have been well documented for both terrestrial plants (Baskin and Baskin, 1998) and for many seagrass species (French and Moore, 2003; Orth et al., in press). Despite the important influences of environmental factors on seed germination, there has been little research in the affects of these parameters on many species of freshwater SAV, including *V. americana* (Barko et al, 1986).

Temperature

Temperature control on production and reproduction have been reported for many terrestrial and aquatic plant species. For example, temperature was found to be the main control on flowering of *Halophila engelmannii* Aschers. (star grass) from Redfish Bay, TX. In controlled laboratory conditions shoots exposed to day lengths of 14 hours and temperatures between 22-24 °C flowered continuously. If temperatures decreased to 18.5 °C or daylight decreased to 12 hours shoots did not flower and remained vegetative (McMillian, 1976). Temperature control of sexual reproduction is also important for *Z. marina* in Chesapeake Bay. Flower production of *Z. marina* occurred when temperatures

increased above 14.3 °C, pollen was released after temperatures reached 16 °C, and seeds were released when temperatures increased to 25 °C (Silberhorn et al., 1983).

In the terrestrial environment, exposure of cultivars (Marzak and Oum-rabia) of the wheat crop species *Triticum durum* Desf. (durum wheat) to increased temperatures (20/15 compared to 28/21; 36/29) resulted in decreased seed vigor and decreased seedling survival (Grass and Burris, 1995). The effects on seed germination were not consistent between cultivars, seeds of Marzak exposed to high temperatures showed decreased germination, whereas high temperatures did not affect seeds of Oum-rabia. Increased temperatures increased time to germination by 2 days (Grass and Burris, 1995). Due to the possible effects of temperature stress on the health of "parent" plants, and consequently production and viability of seeds or clones, it is important to account for temperature throughout the entire growing season.

Temperature serves a large role in seed germination of many aquatic plant species. Temperatures of 15 °C are required for germination of *M. spicatum* (Hartleb et al., 1993); germination of *Stratiotes aloides* L. (water soldier) is significantly less in 11 °C compared to 19 °C (Smolder, et al., 1995); temperatures of 23 – 28 °C initiate germination within a week of exposure for *Hydrilla verticillata* (L.F.) Royle (Hydrilla) (Lal and Gopal, 1993); and increasing temperatures result in increased germination in *Cymodocea nodosa* (Ucria) Aschers. (Cymodocea) with maximum germination at 20 °C (Pirc et al., 1986). A decrease in temperatures also serves as a germination cue for many SAV species. *Zostera capricorni* Aschers. (eelgrass) germination in Australia is initiated when temperatures drop during winter months (Peterken, and Conacher, 1997) and *Z. marina* seeds in England and the United States (Chesapeake Bay) germinate when water

temperatures drop below 15 °C with the greatest amount of germination occurring at temperatures below 6 °C (Moore et al., 1993; Probert and Brenchly 1999).

For emergent plants like *Typha latifolia* L. (broad leafed cattail), large temperature shifts serve as an indication of habitat quality. When seeds were exposed to constant temperatures of 10, 20, and 30 °C all treatments failed to reach 50 percent germination (although germination did increase with temperature). In contrast, when exposed to alternating temperatures of 10/20, 10/30, and 20/30 °C seed germination reached 100 percent. The delay of germination under constant temperature conditions increases the chances of seeds germinating on land in addition to increasing the chance of seedling survival of *T. latifolia* (Lombardi et al., 1997). For submerged species the opposite trend has been observed. *Zannichellia palustris* L. (horned pondweed), a freshwater SAV species, has higher germination (64 percent) at 20 °C compared to alternating temperatures of 10/20 and 20/30 °C (35 percent germination). Final germination rates were also related to temperature with 100 percent germination after at 20 °C (Lombardi et al., 1996). As with the emergent species, temperature may serve as an indicator of habitat quality.

In the limited amount of available research for freshwater SAV, temperature and light have both inhibited and enhanced seed germination depending on the species (Baskin and Baskin, 1998). The effects of temperature, photoperiod, and burial depth on the germination of the freshwater SAV species *M. spicatum* seeds were investigated under controlled laboratory conditions (Hartleb et al., 1993). Temperatures below 15 °C inhibited germination while photoperiod by itself had no effect. The separate effects of

and interactions between light and temperature on *V. americana* seed germination are unknown.

Light

In a study by Muenscher (1936), which investigated the affects of light on germination, photoperiod was shown to have a significant affect on *V. americana* seeds. Intensity of irradiance is an important factor affecting germination, sprouting, and growth. Germination of *V. americana* was inhibited by prolonged exposure to direct light but was not significantly affected by diffuse light (Muenscher, 1936). Kimber et al. (1995) found that wild celery seeds collected from the top 5cm of the substrate in the upper Mississippi River germinated when exposed from 2 percent to 25 percent of the total surface irradiance. The seedlings receiving more than 9 percent of surface irradiance demonstrated continued growth throughout the entire growing season and produced winter buds by November (Kimber et al., 1995). French and Moore (2003) found that *V. americana* plants, grown from tubers, were capable of growth and moderate production when receiving between 2 or 8 percent of available light, with significantly higher production at 28 percent. Plants grown from winterbuds and those grown from seedlings were found to show a similar trend in increasing mortality as available light decreased due to increased turbidity (Doyle and Smart, 2001).

Sediment Composition

Sediment organic content, burial depth, and reduction potential are all factors shown to affect germination and survival of SAV species including *V. americana*. The majority of SAV species are found in sediments with less than 5 percent organic content (Hemminga and Duarte, 2000; Koch, 2001). High organic content in the sediments (> 6 percent) was found to delay germination of *V. americana* 3 – 5 weeks compared to seeds exposed to less organic sediments (Hoover, 1984). Barko and Smart (1986) attributed a decrease in macrophyte survival in sediments with a higher refractory organic content to the inhibition of nutrient uptake. The amplified content of refractory organic material may have increased the concentration of organic acids in the sediment, lowering the overall sediment pH, therefore decreasing the availability of nutrients in the sediments for uptake. This could be a significant environmental control on SAV populations as the primary source of N and P to SAV is from the sediment (Barko et al., 1986). Sediment pH has been found to affect *V. americana* production. A decrease in sediment pH from six to five shortened the growing season of *V. americana* by one month. All plants survived the lower pH conditions, but the production of winterbuds decreased. Those buds that were produced were smaller and less likely to successfully produce healthy rosettes the following growing season. Decreased nutrient availability due to increased acidity was determined to be the primary cause (Grise et al., 1986).

Dissolved Oxygen

Field studies of wild celery tubers have shown that germination decreases with increasing sediment burial depth (Rybicki and Carter, 1986). Tubers collected from

Potomac River, MD sediments at depths shallower than 10 cm showed a 90 percent germination rate. Those collected deeper than 25 cm failed to germinate (Rybicki and Carter, 1986). These results indicate that a sudden large impulse of sediment due to a storm may cause reproductive failure in *V. americana*. One reason for this failure is the increased reduction potential (or increased hypoxia) of the sediments with increased depth (Titus and Hoover, 1991, Terrados et al., 1999). Sediment hypoxia (<1.0 mg l^{-1}; EPA, 2003) can either inhibit or enhance SAV seed germination depending on the species. Hypoxia was shown to completely inhibit germination and negatively affect the health of those seedlings that did germinate by decreasing leaf growth and increasing shoot mortality (Terrados et al., 1999). In contrast, hypoxic conditions increase germination of seagrass species *Z. marina* and *Z. capricorni,* (Moore et al., 1993; Brenchley and Probert, 1998). The effects of hypoxia on SAV germination and survivability have been found to be species specific and warrant further investigation.

Salinity

Salinity is a major limiting factor on SAV species distribution (Moore et al., 2000). In Chesapeake Bay, SAV species are clustered by their location in the estuary, which is usually defined by the salinity regime (Batiuk et al., 1992). Although *V. americana* is a freshwater species, it does have some halo-tolerance. For example, *V. americana* transplants in the Caloosahatchee Estuary (Southwestern Florida) were able to withstand extended periods of increased salinity with an upper bound of 15 psu. Transplants exposed to salinities higher than 10 psu showed reduced growth and survival when compared to transplants at lower salinities (Kraemer et al., 1999). French and

Moore (2003) found that *V. americana* plants exposed to salinities of 10 and 15 psu had lower production and growth rates than those exposed to 0 or 5 psu. Plants exposed to 10 and 15 psu did not flower and demonstrated reduced tuber production. In addition, the effects of salinity interacted with light availability. Stress due to salinity (at salinities greater than 5 psu) may be reduced with increased light availability (French and Moore, 2003). Similar results were found in a study comparing the response of several different euryhaline species, including wild celery, to a range of salinities (0, 2, 4, 6, 12 psu). *Vallisneria americana* was tolerant of the higher salinities but was only able to reproduce asexually (Twilley and Barko, 1990). Although the affects of salinity have been shown to negatively affect reproduction of *V. americana* the affects of increased salinity on the germination of wild celery seeds is largely unknown.

Objectives and Hypotheses

Objectives

The lack of information concerning effects of environmental factors on *V. americana* seed germination is a large void in our knowledge of the basic ecology of this species. As SAV populations decline, it is important to understand the potential impacts of the environment on natural processes of reproduction. In addition, determining which environmental factors affect germination of SAV seeds will increase the efficiency of current restoration practices and provide a mechanism for increased success of current and potential larger scale projects.

The objectives of this study are to observe the changes in environmental conditions in an established *V. americana* bed over an entire growing season (April-

October) and to quantify the effects of similar environmental conditions on *V. americana* seed germination under controlled laboratory conditions. Through this two pronged approach, information on environmental controls of *V. americana* seed germination will be used to synthesize criteria for *V. americana* restoration in Chesapeake Bay. In addition, information gained from this research will provide fundamental information on sexual reproduction in *V. americana* and begin to fill a void on an important portion of this species lifecycle.

Hypotheses

H_1: There will no effect of hypoxic conditions on *V. americana* seed germination compared to oxygenated conditions and no effect of light conditions on *V. americana* seed germination compared to dark conditions.

H_{1a}: Germination of *V. americana* seeds will be enhanced (greater percent germination and less time to germination) in oxygenated dark conditions compared to hypoxic dark conditions.

H_2: There will be no effect of incubation temperatures (13, 22, 25, 29 °C) *V. americana* seed germination.

H_{2a}: Temperatures 13 °C and 29 °C will inhibit *V. americana* germination compared to temperatures in the range of 22 – 25 °C.

H_3: There will be no effect of salinity (0, 5, 10, 15 psu) on *V. americana* seed germination.

H_{3a}: Salinities 5, 10, and 15 psu will inhibit *V. americana* germination compared to salinities of 0 psu.

H_4: Sediment organic matter content (1, 2, 3, 5, 8 percent) and seed burial depth (2, 7, 15, 25, 50, 100 mm) will have no effect on *V. americana* seed germination.

H_{4a}: Sediment containing 5 or 8 percent organic matter and burial depths of 15, 25, 50, or 100 mm will inhibit *V. americana* germination compared to sediments containing 1, 2, or 3 percent organic matter and burial depths of 2 or 7 mm.

H_5: Environmental factors will not interact.

H_{5a}: Interactions between environmental factors will have significant effects on germination of *V. americana* seeds.

METHODS

Site Selection

Three *V. americana* beds were randomly selected in Nanjemoy Creek, Maryland based on historical data and aerial photography (site A- 38° 25.9' N, 77° 87.2' W; site B- 38° 25.9' N, 77° 06.4' W; site C- 38° 26.4' N, 77° 07.1' W; Orth et al., 2003) in order to characterize the population biology of *V. americana* in this system (Figure 3). In addition these sites served as the location of seed collection for subsequent experiments. Nanjemoy Creek is a tidally influenced tributary to the Potomac River, is approximately 14 miles long, and has a "T" shape created by northwestern and northeastern branches that feed the main body of the creek (Figure 3). The majority of the western watershed was residential and or forested.

Site A was located along the western bank of Nanjemoy Creek and contained the largest continual SAV bed of the three sites (Figure 3). Site A also served as the seed pod collection site during October 2003 and October 2004. In addition to *V. americana* other SAV species observed include *Najas* species, *M. spicatum*, and *Hydrilla verticillata.* These species were found throughout the entire bed, to a maximum depth of 77.6 cm below MLLW, although they were not considered to be dominant.

Site B was located on the eastern bank of Nanjemoy Creek directly across from site A (Figure 3). The eastern side of the bed was bounded by a small feeder creek and wetland. Several species of waterfowl were observed during the course of the sampling season. Large reductions in biomass during June were attributed to grazing by these

populations. In addition to *V. americana, Najas* species and *H. verticillata* were observed during May and July-October to a maximum depth of 65 cm below MLLW.

Site C was the northern most sampling site (Figure 3). The site was located along the western bank of Nanjemoy Creek where the shoreline was reinforced with bulkhead. The creek bank along southern edge of the *V. americana* bed was bordered by a small wetland. *Vallisneria americana* was sparsely distributed to a maximum depth of 47.5 cm below MLLW and the only SAV species observed at this site.

Field Sampling

To characterize the mid-bed vegetation at each site, percent cover, shoot density, number of flowering shoots, and leaf length were measured along a transect running from shoreline to the outer edge of the SAV bed. Methods used for this characterization closely followed protocols developed by the National Estuarine Research Reserve System SAV monitoring program and the Seagrass Watch seagrass monitoring system (McKenzie et al., 2001). A 0.5 m^2 PVC square was randomly tossed within a 2 m^2 area 3 times every 10 meters. Each time percent cover and shoot/flowering density within the sampling square were estimated. Percent cover within the square was visually measured and shoot/flowering shoot density was estimated by counting the number of *V. americana* shoots/flowering shoots within a 20 cm diameter ring placed within the PVC square. The number of shoots was corrected for the area of the ring. In addition, the longest leaf within the diameter of the ring was measured. The number of seed pods located within the ring was also counted, and the number of individual seeds within the seed pods were counted and tested for viability using the tetrazolium method due to its increased

accuracy and time efficiency over traditional germination tests (Lakon, 1949; AOSA 1970). Seed embryos were removed from their seed coats and soaked in a 1 percent tetrazolium chloride (tetrazolium) solution for 24 hours before examination on a dissecting scope at 10x magnification.

To determine the mid-bed biomass at each site, a 22 cm diameter corer was inserted into the sediment at three random locations dispersed throughout the mid-portion of each bed. The mid-portion of the bed was defined as the area between 40-80 m for sites A and B and 20-40 m for site C. The entire core was sieved in the field through a 1 cm mesh screen. Entire plants, winterbuds, and all extra plant tissue (leaves) were collected in plastic bags and stored on ice until taken back to the lab and analyzed. During lab analysis all plants were sectioned into above and below ground material with separation occurring at the basal meristem. The longest leaf length and the average leaf area were measured from each sample using a Li-Cor 3100 leaf area meter. To determine where plant resources were allocated during the growing season, both the above and belowground samples were dried in pre-weighed aluminum envelopes for 5 days at 50 °C or until a constant dry weight was reached.

Sediment Characterization

Three sediment cores (11.4 cm diameter, 20 cm length) were taken from each site in April, sectioned into slices of 0-2, 2-5, 5-10, 10-15, 15-20 cm horizons, and sieved (63 μm sieve), washing slit and clay fractions into a graduated cylinder. After 24 hours, pipette analysis was performed to determine the clay (8 phi) and silt (4 phi) fractions of the sample. Dry weights of the aliquots were compared and percent sand, silt, and clay

fractions were determined (modification of Plumb, 1981). All sediment was classified based on sand silt clay ratios (Shepard, 1954).

Three sediment cores (11.4 cm diameter, 20 cm length) were collected from each site in April, June, July, September, and October. All three cores were sectioned as described previously and quartered. Two quarters were rinsed with de-ionized (DI) water through a 0.5 mm sieve. All *V. americana* seeds were collected, counted, and analyzed for viability using a tetrazolium adsorption method (AOSA, 1970). Percent organic matter in the sediment was determined by drying a second quarter of the sediment sub-sample at 60°C for 5 days or until a constant dry weight was reached. After the samples were cooled in a desiccator, the sediment was weighed and combusted at 500°C for 5 hours. The sample was weighed again and percent organic matter was calculated (Erftemeijer and Koch, 2001).

A third quarter of the sub-sample was analyzed to determine sediment exchangeable nutrients. The section was extracted in 2 M KCl, shaken for 1 hour, centrifuged, filtered (Gelman Supor, 0.45 μm), and frozen until analyzed. DIN and DIP was determined in the samples using a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002).

Water temperature (°C), salinity (psu), dissolved oxygen (mg l^{-1}), and pH were measured with a Yellow Spring Instruments, Inc. (YSI, Inc., Yellow Spring, Ohio) model 650 sonde at each site. In addition, three water samples were collected from each site. The samples were analyzed for DIP and DIN as described previously. The remaining water samples were filtered and analyzed for chlorophyll *a* (Strickland and Parson, 1972) and total suspended solids (TSS). TSS was quantified from a well-mixed sample of

known volume. The sample was filtered through a GF/F filter and the residue retained on the filter was dried to constant weight at 103 – 105 °C and reported as mg total suspended solids l^{-1}. Ambient irradiance was measured with a Li-Cor terrestrial sensor (LI-190SA) and light attenuation, or K_d (m^{-1}), to a depth of 1 m through the water column was quantified by measuring light levels beneath the water surface at each site with a Li-Cor underwater light sensor (LI-192SA).

Seed Pod Collection

Seed pods were collected by hand in late October 2003 and again in October 2004 from site A in Nanjemoy Creek prior to seed pod release. Once collected, the seed pods were stored in plastic bags filled with creek water, placed on ice, and transported back to the lab. The seed pods were then transferred to plastic containers, sealed, and stored at 4-6 °C until analysis (Baskin and Baskin, 1998). To remove seeds, individual seed pods were transferred to glass trays where the pods were sectioned down the middle with a razor blade. The seeds were removed by tweezers and rinsed in DI water.

Germination

Seed germination during all experiments was defined as the emergence of the seed embryo from the seed coat (AOSA, 1981). To quantify time to germination and total number of germinated seeds, the number of germinated seeds was counted daily and recorded. Time to germination was calculated by recording the number of seeds that germinated per day per treatment and analyzed using survival analysis techniques (The SAS® System for Windows, SAS Institute, Inc.). At the end of all experiments all

seedlings were removed and measured for length. The seedlings were dried for 5 days at 50 °C and weighed again to calculate dry weight. The viability of the remaining ungerminated seeds was tested with the tetrazolium adsorption method. All germination experiments included at least three replicates of 50 seeds or more based on preliminary germination experiments and a literature review from Baskin and Baskin (1998).

Oxygen and Light Experiment

To observe the affects of oxygen and light on germination of *V. americana* seeds, 5 replicates of 50 seeds were placed in 250 ml sealed serum bottles in either oxygenated-light, oxygenated-dark, hypoxic-light, or hypoxic-dark conditions (20 bottles total; Figure 4). Serum bottles were randomly placed under diffusive light (< 160 μmol m^2 s^{-1}) in an environmental growth chamber (EGC, Inc.). The conditions in the environmental growth chamber (EGC) were set to a 12-hour photoperiod; temperature controlled to 22 ± 2 °C, and humidity at 12 percent. To screen for a chamber effect, light levels were monitored using a Li-Cor terrestrial sensor (LI-190SA).

Two 5-gallon carboys were filled with DI water. One carboy was then bubbled with nitrogen gas to remove oxygen. Dissolved oxygen (DO) levels in both carboys were measured with a YSI, Inc., DO meter (Model 85). DO concentrations for oxygenated treatments were 8.00 mg l^{-1} and 0.29 mg l^{-1} for the hypoxic treatments. Serum bottles for the dark experiment were completely covered in electrical tape with removable strips placed along the bottom to allow daily germination counts. Fifty seeds were placed into each serum bottle; bottles were filled with the appropriate water type, and then sealed with a rubber stopper and metal cap. For the 10 oxygenated serum bottles, a 20-gauge

1½ " needle attached to a rubber hose and air pump was inserted through the rubber cap to bubble in air. A 23-gauge ¾ " needle was then be inserted to let excess air out of the bottle.

Daily germination counts were recorded to quantify time to germination and percent germination in each bottle. To count the seeds in the dark serum bottles all lights in the EGC were shut off and an 80-watt red wavelength light was used for germination counts. The short exposure to red light allowed germination counts without exposing the seeds in the dark treatment to extended periods of useable wavelengths of light. After 21 days the seeds and seedlings were removed and counted.

Temperature Experiment

To determine the affects of temperature on germination of *V. americana* seeds, 4 replicates of 50 seeds were randomly placed in petri dishes located in individual 110 liter aquaria with temperatures of 13, 22, 25, and 29 °C (Figure 5). One petri dish was placed in each aquaria (16 aquaria total) and one aquarium equaled one replicate. Seeds were placed in 5 rows of 10 with identical orientation to the substrate. The seeds were then covered by a 9.0 cm diameter Whitman GF/F filter and secured. Each petri dish was then placed in individual aquaria filled with equal volumes of DI water and covered with a thin piece of glass to reduce evaporation. The water in all of the aquaria was bubbled. The tanks were randomly assigned a temperature depending on the treatment using a random number generator and placed in the EGC. The treatment temperatures were maintained with mini-thermal compact aquaria heaters (50 watts) and monitored daily.

The temperature within the chamber was maintained at 7.0 °C with a photoperiod of 12 hours. Light levels were maintained at or below 150 μmol m^{-2} s^{-1}.

Seed germination in the individual petri dishes was monitored daily for 14 days. In addition, water temperature (°C), dissolved oxygen (mg l^{-1}), and salinity (psu) was measured with a YSI, Inc. (model 650 sonde) and recorded daily. To quantify any potential chamber effects, light was measured just above the surface of the water for each tank with a Li-Cor terrestrial sensor (LI-190SA). Potential tank effects were quantified by measuring light levels just below the water's surface in each tank with a Li-Cor underwater light sensor (LI-192SA). Water samples were collected from each tank before and after the sampling period. The samples were filtered (Gelman Supor, 0.45 μm) and frozen until analyzed for DIP, DIN, and DON as directed above.

Salinity Experiment

The affects of salinity on germination of *V. americana* seeds were tested with 4 replicates of 50 seeds at 0, 5, 10, and 15 psu. The petri dish design was identical to the one used in the temperature experiment. The experiment used four 1.22 m x 2.44 m tanks in the SAV greenhouse at the Virginia Institute of Marine Science (VIMS), Gloucester Point, Virginia (37° 14.8' N, 76° 30.3' W). There were four aquaria each containing one petri dish with 50 seeds in each large tank (Figure 6). Each tank was set up with a separate recycling freshwater system filtered with a Tarpon Lifeguard sand filter system, UV filter, and chilled with a Pacific Coast 1 hp chiller to a temperature between 20 – 23 °C.

For 34 days, daily germination counts were recorded to quantify time to germination and percent germination per petri dish. All tanks were monitored daily for temperature, DO, and salinity with a YSI, Inc. 650 sonde. Ambient light levels were measured with two Li-Cor terrestrial sensors (LI-190SA) located in-between the three tanks. The data were recorded with a LI-1000 data logger and integrated over 15 minute intervals throughout the entire sampling period. Potential tank effects were quantified as described previously.

Sediment Composition and Burial Depth Experiment

Sediment from site C was collected in the field and stored in 5 gallon buckets at temperatures ranging from 7 – 9 °C for the sediment type burial depth experiment (STBD). To develop a range of sediment composition, sediment was collected from the near-shore (NS) region and from the deepest edge (DE) of the bed. All sediment was sieved through a 0.5 mm sieve to remove any seeds and then homogenized. The sediment was mixed into treatments consisting of 100 percent NS; 75 percent NS: 25 percent DE; 50 percent NS: 50 percent DE; 25 percent NS: 75 percent DE; and 100 percent DE. Sediments were analyzed for percent organic content, exchangeable nutrients (DIN and DIP), and percent sand silt clay and characterized based on sand, silt, clay ratios as described previously. The sediment mixture for each treatment replicate was placed in a 102 mm diameter PVC tube (120 mm x 120 mm) that was capped on the bottom. Fifty seeds in 5 rows of 10 were buried at depths of 2, 7, 15, 25, 50, or 100 mm in the artificial core. Each core was replicated three times. All cores were placed in one of three tanks (1.2 m x 2.4 m) in the SAV greenhouse (30 samples per tank) based on a

complete block design (Figure 7). Each tank was filled with 550 gallons of freshwater that was maintained at a temperature range between 23 – 25 °C using a Pacific Coast 1 hp chiller. Tank water was filtered with a Lifeguard Tarpon sand filter and a UV filter to remove any suspended organic material.

Daily germination counts were recorded for 31 days. All tanks were monitored daily for temperature, DO, and salinity with a YSI, Inc. 600 data sonde. Ambient light levels and potential tank effects on irradiance were quantified as described previously. Randomly chosen samples from each tank, representative of each sediment type, were measured for nutrient content (DIP, DIN) once before, during, and after the completion of the experiment. Vertical redox (Eh) profiles were measured with a 51 cm platinum electrode for all sediment types. The probe was inserted into the top of the core and redox was measured every 5 mm. Final readings were corrected for temperature relative to the platinum electrode (Hinchey and Schaffner, 2005).

Statistical Analyses

Treatment effects of each variable in the individual experiments were determined using Analysis of Variance (ANOVA; StatView for Windows, SAS Institute Inc.). All experiments were set up using a randomized, complete block design. Prior to analysis, all percent data were arcsine square root transformed. Normality was confirmed visually, and homogeneity of variance verified with Cochran's Test. Transformations did not affect the interpretation of data; thus non-transformed means are presented.

Survival analyses using the LIFETEST system were preformed on time to germination data for all experiments (The SAS System for Windows, SAS Institute Inc.).

Survival analysis was selected due to large amount of right-censored data characteristic of germination experiments (Scott et al., 1984). Seed data was censored if germination did not occur and non-germinated seeds were flagged as censored values prior to analysis. The LIFTEST program allows for right censored data (i.e. seeds that did not germinate prior to termination of experiment) during analysis of survivability. Results are presented in mean time to germination (MTG).

Field site characteristics were analyzed graphically. To quantify the effects of time on bed development and sediment characteristics, normality was confirmed visually, and homogeneity of variance verified with Cochran's Test. The effect of time on SAV bed characteristics, sediment characteristics and water quality was determined using ANOVA (ANOVA; StatView for Windows, SAS Institute Inc.). Seed bank characteristics were compared to environmental factors using regression and correlation techniques.

Criteria Development

"Optimal" environmental conditions for *V. americana* seed germination were established based on the statistical analysis of all experimental data and field site characterizations. Environmental conditions that produced the shortest time to seed germination, the greatest germination percentage, and most resembled seasonal field conditions (for example, water temperatures that occur in freshwater areas in April or May) were considered optimal.

RESULTS

Vallisneria americana Bed Characterization

Environmental Conditions

Water Temperature

Temperatures increased from minimum temperatures of approximately 15 °C in April to maximum temperatures of 31 °C in August before declining again to 18 °C in October (Figure 8). All sites had similar water temperatures with an average temperature of 26 °C at site A, 25 °C at site B, and 25 °C at site C (App. 1).

Dissolved Oxygen

Dissolved oxygen (DO) levels in Nanjemoy Creek fluctuated throughout the growing season but never fell below 6.34 mg l^{-1}. For site A DO ranged from 8.93 – 11.44 mg l^{-1} with an average of 10.01 mg l^{-1}; concentrations at site B ranged from 8.71 – 11.83 mg l^{-1} with an average of 10.14 mg l^{-1}; and site C had the lowest DO concentrations that ranged from 6.84 – 9.43 mg l^{-1} with an average of 8.15 mg l^{-1} (App. 1).

Salinity

Salinity in Nanjemoy Creek never exceeded 5 psu between April and October 2004. Salinities increased from lows in April (1.33 psu sites A and C; 1.55 psu site B) to highest recorded concentrations in July (3.84 psu site A; 4.29 psu site B; 3.56 psu site C). The average salinity at site A was 2.68 psu, 2.93 psu for site B, and 2.54 psu for site C (Figure 9; App. 1).

Light

Water clarity, based on light attenuation or K_d (m^{-1}) values to a depth of 1m, varied per site. The most protected location with the greatest extent of SAV cover, site A, had a range of K_d values from 0.8 to 2.4 m^{-1} with a geometric mean of 2.0 m^{-1}. K_d in site B ranged from 1.5 to 3.6 m^{-1} and a geometric mean of 3.6 m^{-1} while K_d at site C ranged from 1.2 to 2.2 m^{-1} with a geometric mean of 4.5 m^{-1} (App. 1). K_d values of < 3.6 m^{-1} to 1 m are required to meet the established SAV habitat criteria for freshwater species in Chesapeake Bay (EPA, 2003). All sites were within this threshold throughout the 2004 growing season.

Chlorophyll a

Water-column chlorophyll *a* concentrations varied both spatially and temporally. Site B had the highest mean chlorophyll *a* concentration of 26.32 $\mu g\ l^{-1}$ and concentrations ranged from 9.0 – 43.0 $\mu g\ l^{-1}$. Site A had the second highest mean chlorophyll concentration, 19.2 $\mu g\ l^{-1}$, and the greatest range in concentrations from 9.8-54.5 $\mu g\ l^{-1}$. Site C had the lowest chlorophyll concentrations and was similar to site A with average chlorophyll *a* levels of 18.6 $\mu g\ l^{-1}$ with concentrations ranging from 11.7-34.9 $\mu g\ l^{-1}$ (App. 1).

Total Suspended Solids

The concentration of total suspended solids (TSS) in the water column also varied spatially and temporally. Site C, located furthest away from the mouth of Nanjemoy Creek, had the highest mean TSS concentrations of the three sites with 26.8 $mg\ l^{-1}$ and a

range of 17.3-35.6 mg l^{-1}. Site A had the largest range in TSS concentrations from 8.8-38.3 mg l^{-1} with a mean concentration of 13.3 mg l^{-1}. Site B had the lowest mean water column TSS concentration of 13.3 mg l^{-1} and a range of 3.8-23.0 mg l^{-1} (App. 1).

Nutrient content

Concentrations of NO_x (NO_2^- + NO_3^-), ammonium (NH_4^+), and orthophosphates (PO_4^{-3}) were significantly different for all sites over time (App. 3). Increases in NO_2^- + NO_3^- concentrations occurred in all sites between September and October. Site A increased from 0.1 –16.1 μM, site B from 3.4-17.5 μM, and site C from 0.1 – 29.1 μM (App. 2). NH_4^+ and PO_4^{-3} also showed a slight increase (less than 1 μM for all sites) between September and October, however the increase was not as large (App. 2). September and October were also the months of maximum nutrient concentrations for all sites (with the exception of NH_4^- concentrations at site C which peaked in July; App. 2).

Sediment Characteristics

Sediment Classification

Based on sand, silt, and clay ratios, the sediment of sites A and B were classified as both clayey sand and sediment at site C as sand (Table 1). Sediment ratios did not vary significantly with depth ($F = 0.01$, $n = 3$, $p = 0.9895$ site A; $F = 0.07$, $n = 3$, $p = 0.9339$ site B; $F = 0.13$, $n = 3$, $p = 0.8809$ site C) at any site (App. 5).

Organic Content

Organic content (percent lost on ignition) of the sediments did not vary significantly with depth at any site (App. 5). Sediment organic content did not significantly vary over time for sites B ($F = 0.73$, $n = 5$, $p = 0.6740$) or C ($F = 2.22$, $n = 5$, $p = 0.6312$); however, time was a significant factor for site A ($F = 1.35$, $n = 5$, $p = 0.0001$; App. 4). Organic matter increased by approximately 55 percent in site A between July and August from 4.4 to 6.9 percent (Table 1). Similar trends were observed in sites B and C, but these increases were not significant (App. 4). Site A had the largest range in organic content from April to October increasing from 1.2 – 6.9 percent between April and July before decreasing to 2.2 percent in October (Table 1). Sites B and C had smaller changes in concentrations with ranges from 1.2 – 2.5 percent between April and July before returning to 1.4 percent in October for site B and 1.0 – 1.9 percent, April to July decreasing to 1.4 percent in October for site C (Table 1). Maximum concentrations in organic content for all sites were recorded in August (Table 1).

Nutrient content

Sediment nutrient concentrations NO_x ($NO_2^- + NO_3^-$), ammonium (NH_4^-), and orthophosphates (PO_4^{-3}), fluctuated over time and depth for all sites. $NO_2^- + NO_3^-$ concentrations were significantly different over time for all sites (site A: $F = 5.79$, $n = 5$, $p=0.0014$; site B: $F = 3.50$, $n = 5$, $p=0.0186$; site C: $F = 8.66$, $n = 5$, $p=<0.0001$; App. 7). $NO_2^- + NO_3^-$ increased from levels below detection limits in April to maximum concentrations of 31.35 μM (site A) and 34.34 μM (site B) in September. Concentrations of $NO_2^- + NO_3^-$ in site C increased from minimum levels in April (below detection limits)

to maximum concentrations of 4.38 μM in October. NO_2^{-2} + NO_3^- concentrations also varied significantly over depth for all sites (site A: F = 10.65, n = 3, p=0.0003; site B: F = 11.20, n = 3, p=0.0002; site C: F = 8.10, n = 3, p=0.0015; App. 7). Concentrations were greater in the 0 – 5 cm layer than the 5 – 10 cm layer (App. 6).

NH_4^+ concentrations were consistently greater at site A for all depths from April through October with the exception of the 0 – 2 cm depth in October when both sites B and C had greater concentrations (App. 6). Date was a significant factor for both sites A (F = 8.48, n = 5, p=0.0001) and C (F = 4.57, n = 5, p=0.0053) and depth as a factor was only significant for site A (F = 10.47, n = 5, p=0.0004; App. 7). Maximum NH_4^+ concentrations (0 – 10 cm) occurred in August for sites A (130.68 – 259.18 μM) in September for site B (63.23 – 56.89 μM) and in October for site C (57.81 – 79.81 μM, App. 6). The largest increase in NH_4^+ concentrations occurred when they vaulted from 103 μM to 306 μM at depths over 10cm for site A in July (App. 6). Although concentrations varied over time for all sites, they never exceeded the expected maximum NH_4^+ concentrations from Potomac River sediments (Carter et al., 1987).

PO_4^- concentrations did not change significantly over depth or time for sites B and C, but were substantially greater at depths >5cm at site A (App. 6). Maximum concentrations over all depths occurred in August for all sites and ranged from 0.74 μM (site A) to 0.23 μM (site B; App. 6). PO_4^- levels never exceeded 1 μM at any site over any depth during the entire observation period (App. 6).

Redox

Measurements of oxidation-reduction potential, or Eh, in the sediments were measured in cores collected in April 2004. Eh was similar at all sites throughout the top 5 cm of sediment and decreased with depth. Eh ranged from 164 mV (at the sediment surface) to –39 mV (depth of 5 cm) for site A; 268 mV –38 mV site B, and 263 mV-40 mV for site C (Figure 10).

Vegetation Characteristics

Biomass

Biomass (both above and below ground) increased from April, when the first shoots were produced, to maximum biomass between July and September when flowers and seed pods were produced above and below ground plant material then decreased in October (Figure 11).

Total plant biomass of *V. americana* was the greatest at site A and ranged from 39.79 – 314.24 g DW m^{-2} with a mean of 195.50 ± 43.22 g DW m^{-2} (Table 2). Site B produced the second largest amount of biomass ranging from 11.46 – 377.48 g DW m^{-2} with a mean of 171.37 ± 58.16 g DW m^{-2} (Table 2). Site C produced the smallest amount of biomass out of all sites for all months (except for September and October when sites A and B had smaller amounts respectively). Biomass at site C ranged from 1.36 – 522.62 g DW m^{-2} with a mean biomass of 148.58 ± 72.17 g DW m^{-2} (Table 2).

Vallisneria americana above and below ground biomass followed similar trends throughout the entire 2004 growing season (App. 8). For the majority of the growing season, site A had the greatest amount of above ground biomass with sites B and C showing similar results. The amount of above ground biomass ranged from 11.28 –

140.19 g DW m^{-2} with a seasonal average of 84.00 ± 15.78 g DW m^{-2} for plants in site A (Figure 12). At site B above ground biomass ranged from 1.94 – 146.34 g DW m^{-2} with a seasonal average of 66.69 ± 26.06 g DW m^{-2} (Figure 12). The amount of above ground biomass in site C ranged from 0.31-339.45 g DW m^{-2} with a seasonal average of 98.40 ± 50.72 g DW m^{-2} (Figure 12). Below, ground biomass ranged from 7.81 – 147.67 g DW m^{-2} in site A; 9.52 – 84.80 g DW m^{-2} for site B, and 1.05 – 183.16 g DW m^{-2} for site C (Figure 12). Site A had the largest mean below ground biomass with 56.71 ± 16.74 g DW m^{-2}, followed by site C with 49.47 ± 10.45 g DW m^{-2}, and site B with 52.80 ± 27.10 g DW m^{-2} (Figure 12).

Percent Cover

Overall mid-bed cover (40 – 80 m) by *V. americana* was similar in sites A and B with seasonal means of 71 ± 7 percent and 75 ± 7 percent respectively. Mid-bed cover (20 – 40 m) at site C was sparse and patchy. The seasonal mean for cover at site C was 34 ± 9 percent. Percent cover ranged from 4 – 94 percent m^{-2} at site A, 18 – 94 percent for site B, and 13 – 46 percent for site C (Table 3).

Density

In 2004, mid-bed density (shoots m^{-2}) of *V. americana* increased from June to August for all sites. The density of shoots increased from 53 shoots m^{-2} in June to 227 shoots m^{-2} in August at site A; site B increased from 78 to 267 shoots m^{-2} and site C increased from 21 to 106 shoots m^{-2}. Site B had the greatest mean density (210 ± 14 shoots m^{-2}) followed by site A (160 ± 14 shoots m^{-2}) and site C (74 ± 19 shoots m^{-2};

Table 4). Data were not collected in April, May, September, and October due to weather conditions.

Leaf Length

Leaf length increased with increasing water depth and time for all sites. Leaf length increased from April to August (or September for site C) and then declined again until October. Leaf length ranged from 37-106.8 cm for site A, from 10.6-108.4 cm for site B, and from 4.7 – 30.7 cm for site C. Mean leaf length was greatest at site A (93.8 ± 4.5 cm), decreased to 73.4 ± 4.8 cm for site B, and was shortest at site C (15.7 ± 2.8 cm; Table 5).

Seed Production, Abundance and Viability

Seed Production

The abundance of *V. americana* flowering shoots was highly variable at all sites during the months of August and September. On average in August and September, site A had 220 shoots m^{-2}, 33 percent of which were flowering (72 flowering shoots m^{-2}). Site B had 260 shoots m^{-2} on average with mean flower production of 43 percent (112 flowering shoots m^{-2}). The mean density of shoots at site C was 74 shoots m^{-2}, 30 percent (22 shoots m^{-2}) of which flowered. Approximately half of the flowers collected were female. Of the total pods collected the mean number of seeds per pod was 150. Therefore, on average, between 4,500 and 16,000 seeds m^{-2} were produced depending on site (Table 6).

Seed Abundance

Few *V. americana* seeds were collected from monthly sediment cores. The abundance of seeds did not vary with depth or time, therefore all seed data are reported as cumulative counts from each core. The last seed collection was in September because the 2004 growing season seeds were not yet released. Based on tetrazolium staining, the majority of seeds collected from Nanjemoy Creek sites were non-viable. Of the seeds produced at Site A 0.5 percent were viable and at site B 0.6 percent were viable. Site C did not produce any seeds. There was little presence of a *V. americana* seed bank at the Nanjemoy Creek sampling sites (Table 6).

Experimental Results

Environmental Conditions

Temperature

Temperature was maintained within the range of 22-25 °C for all experiments where temperature was not the variable being examined (App. 9 – 11). Water temperatures for the dissolved oxygen experiment were not recorded within the bottles because the bottles were sealed; however, the EGC temperature was maintained at 23 ± 1 °C for the duration of the experiment. Water temperature in all tanks were maintained at 25 ± 1 °C during the sediment type and burial depth (STBD) experiment (App. 11) and during the salinity experiment (App. 10). During the temperature experiment, temperatures were maintained at 4 treatment levels; 13 ± 1 °C, 22 ± 1 °C, 25 ± 1 °C, and 29 ± 1 °C (Table 7; App. 9).

Dissolved Oxygen

Dissolved oxygen (DO) concentrations were maintained at saturated levels for all experiments and treatments where dissolved oxygen was not the variable being examined (App. 9 – 11). DO levels were not recorded during the course of the dissolved oxygen experiment because the bottles were sealed; however the initial DO concentration in the oxygenated treatment was 8.00 mg l^{-1} and DO was 0.29 mg l^{-1} for the hypoxic treatment. DO concentrations ranged from 6.9 ± 0.1 mg l^{-1} to 10.7 ± 0.3 mg l^{-1} across all temperature treatments (App. 9). During the STBD experiment, DO levels were 8.3 ± 0.2 mg l^{-1} (App. 11) while during the salinity experiment DO levels were 7.8 ± 1 mg l^{-1} (App. 10).

Salinity

Salinity values were maintained at less than 1 psu for all experiments and treatments where salinity was not the variable being examined (App. 9-11). Salinity levels were not recorded during the course of the dissolved oxygen experiment because the bottles were sealed; however, initial salinities were less than one psu. During the salinity experiment treatment levels were maintained at 4 separate treatment levels; 0.13 ± 0.02 psu, 5.24 ± 0.09 psu, 10.50 ± 0.10 psu, or 15.49 ± 0.17 psu (Table 8, App. 10).

Light

Light levels were maintained at approximately 160 μmol m^{-2} s^{-1} during the oxygen and light and temperature treatments. Light levels during the sediment type burial depth and salinity experiments fluctuated with natural irradiances and were not substantially different between tanks or treatments.

Nutrient Content

Water column NO_x (NO_2^- + NO_3^-), ammonium (NH_4^+), and orthophosphates (PO_4^{-3}), were variable for all experiments but never exceeded typical ambient levels observed in Nanjemoy Creek. Nutrient concentrations were not measured for the dissolved oxygen experiment due to loss of water after the seal was removed. NO_2^- + NO_3^- concentrations were significantly different between STBD treatments ($F = 3362.15$, $n = 3$, $p = <0.0001$; App. 14) but did not vary significantly between temperature treatments ($F = 0.82$, $n = 4$, $p = 0.4971$; App. 13). NO_2^- + NO_3^- concentrations were less than 2 μM for both the temperature and STBD experiments and therefore not considered to bias any final data (App. 13 and 14). NO_2^- + NO_3^- concentrations were also significantly different during the salinity experiment where concentrations increased from 0.0 to 12.3 μM with increasing salinity ($F = 491.24$, $n = 4$, $p = <0.0001$; App. 12). This was attributed to the mixing of filtered seawater with DI water to achieve the higher salinity treatment levels. By the end of the experiment all NO_2^- + NO_3^- concentrations were less than 2 μM (App. 12).

Ammonium concentrations increased significantly over time during the temperature experiment ($F = 11.30$, $n = 2$, $p = 0.0026$; App. 13). The difference in NH_4^+

was not different between experiments, therefore the increase was similar across all treatments and any potential effect did not influence the final results of the experiment. The final concentration of NH_4^+ in the water column for all treatments did not exceed 40 μM and was not considered to cause any bias in the final results (App. 13). NH_4^+ concentrations in the salinity experiment did not vary with time or across treatments (App. 12). Concentrations of NH_4^- were significantly different between tanks during the STBD experiment ($F = 16.92$, $n = 3$, $p=0.0034$). However, concentrations never increased above 1 μm and were not considered to bias any results (App. 14).

PO_4^{-3} concentrations were not significantly different between treatments for the temperature ($F = 1.48$, $n = 4$, $p = 0.2461$; App. 13) and STBD experiments ($F = 0.27$, $n = 3$, $p=0.7749$; App. 14). Water column PO_4^{-3} concentrations ranged from 17.34 – 17.46 μM during the STBD experiment, which is an order of magnitude greater than concentrations for all other experiments. The increased concentration of PO_4^{-3} may be attributed to the experimental system; however, concentrations were elevated in all three tanks and therefore were not thought to confound any results (App. 14). Results from the salinity water column nutrient data indicate that PO_4^{-3} was significantly different between treatments and over time ($F = 100.97$, $n = 4$, $p = <0.0001$; App. 12). Although concentrations of PO_4^{-3} did increase between salinity treatments, concentrations ranged between 0.2 μg –1.0 μg and therefore were not considered to bias the final results of the experiments (App. 12).

Sediment Characteristics

Percent Organic Content

All sediment type treatments in the sediment type and burial depth experiment (STBD) were substantially different (Table 9). The organic content of sediment during the STBD experiment ranged from 1 to 8 percent (Table 9) The 0 percent NS sediment treatment contained 8.3 ± 0.2 percent organic content, 25 percent NS treatment contained 4.8 ± 0.0 percent organic matter, 50 percent NS treatment contained 2.8 ± 0.0 percent organic matter, the 75 percent NS treatment contained 1.7 ± 0.0 percent organic matter, and the 100 percent NS treatment contained 0.9 ± 0.1 percent organic content (Table 9).

Sediment Classification

All treatments in the STBD experiment also varied in percent sand:silt:clay ratios. The percentage of sand in the treatment sediments increased proportionally with the amount of near shore sediment content within the treatment (Table 9). The percentage of sand in treatment sediments ranged from 3 ± 0.2 percent (0 percent NS) to 86 ± 0.3 percent (100 percent NS). Concentrations of silt and clay decreased proportionally with an increase in the near shore sediment content within the treatments. Silt content decreased from 37 ± 2.5 percent (0 percent NS) to 4 ± 0.3 percent (100 percent NS) and clay content decreased from 60 ± 2.4 percent (0 percent NS) to 9 ± 0.6 percent (100 percent NS; Table 9). Based on Shepard's Classification the 0 percent NS treatment was silty clay, 25 percent was mixed sediments, 50 and 75 percent were clayey sands, and the 100 percent NS treatment was sand.

Redox

Measurements of the oxygen reduction potential of the treatment sediments suggested that redox did not vary among sediment types. These results were similar to the measurements collected from the Nanjemoy sediment cores. Although sediment type and organic content varied among the different treatments, redox values were similar from 2 mm to a depth of 100 mm (Figure 13).

Sediment Nutrient content

Sediment porewater NO_x (NO_2^- + NO_3^-) levels was below detection limits (i.e. <0.01 μM) for all treatments (App. 15). Ammonium concentration increased with decreasing percent NS sediment content and between sediment types. NH_4^+ concentrations ranged from 269.5 ± 90.1 – 405.6 ± 24.0 μM, and were not significantly different ($F = 0.51$, $n = 4$, $p=0.6910$; App. 15). There was significantly more PO_4^{-3} in the 100 percent NS treatment sediments compared to all other treatments ($F = 8.94$, $n = 4$, $p=0.0124$). PO_4^{-3} concentrations were 4 times greater compared to the other treatments and concentrations averaged 3.0 ± 0.7 μM for the 100 percent NS treatments. PO_4^{-3} concentrations for the remaining treatments ranged from 0.4 ± 0.0 percent (50 percent NS) to 0.6 ± 0.0 percent (25 percent NS; App. 15).

Description of Seeds and Seedlings

Seedling Weight

Seedling weight at the conclusion of individual experiment was not significantly different among any treatments for that experiment. Seedling weight was not different

after the DO experiment. Average seedling biomass was similar for both the salinity and temperature experiments. For the salinity experiment mean weight was 0.0008 ± 0.0001 g shoot^{-1} and for the temperature experiment mean seedling weight was 0.0005 ± 0.0002 g shoot^{-1}. For the STBD experiment average weight was 3 orders of magnitude greater than the other experiments. Average seedling weight for the STBD experiment was 0.50 ± 0.03 g shoot^{-1}.

Seed Viability

The number of viable seeds collected at the end of the salinity experiment increased with increasing salinity. Out of 200 seeds exposed to the 0 psu treatment 39 did not germinate and, on average, only 21 ± 8 percent of those remaining seeds were still viable. The number of non-germinating seeds and the overall viability of the remaining seeds increased slightly in the 5 psu treatment. In this treatment 43 seeds did not germinate and 31 ± 8 percent of the remaining seeds were still viable. For the 10 and 15 psu treatments the number of non-germinated seeds increased to 48 and 42 seeds respectively with 54 ± 4 percent and 53 ± 6 percent of these seeds retaining viability. As the number of non-germinating seeds increased with salinity the viability of the remaining seeds also increased (Table 10).

The number of viable seeds collected at the end of the temperature experiment decreased with increasing temperature. On average, 3 percent of the seeds in the 13 °C treatments germinated. Of the remaining seeds 90 ± 1 percent were still viable, with a mean of 44 viable seeds remaining per replicate or 175 total viable seeds out of 200 at the conclusion of the experiment. During the 22 °C treatment 75 ± 10 percent of the seeds

germinated and 77 ± 6 percent of the remaining seeds were still viable. Therefore, out of the 200 seeds exposed to 22 °C approximately 40 viable seeds remained at the end of the experiment. Similar results are shown with the 25 and 29 °C treatments. The numbers of non-germinated seeds (out of 200) exposed to 25 and 29 °C temperatures were 7 and 2 respectively. Only 2 seeds remained viable from the 25 °C treatment and none from the 29 °C treatment (Table 11).

The number of viable seeds did not change with the proportion of near shore sediment (percent sand) mixed into the treatment. Viability of the remaining non-germinated seeds ranged from 52 – 65 percent with an average viability of 60 percent (Table12).

Percent Germination and Time to Germination

Dissolved Oxygen and Light

The presence of oxygen significantly increased germination compared to hypoxic treatments ($F = 8.72$, $n = 2$, $p = 0.0094$), while light/dark treatments had no effect on germination of *V. americana* seeds ($F = 0.00$, $n = 2$, $p = 0.9676$; Table 13). Due to the lack a light effect on germination, data were analyzed with the presence/absence of oxygen as the sole factor. Germination increased from total germination percentages of 45 percent under hypoxic conditions to 75 percent under oxygenated conditions (Figure14).

The negative effect of hypoxic conditions on germination of *V. americana* seeds was also reflected in an increased overall mean time to germination (MTG) between

oxygenated and hypoxic conditions. Seeds under oxygenated conditions had a mean time to germination of 11 days compared to 13 days for seeds under hypoxic treatments.

Salinity

Germination of *V. americana* seeds was significantly influenced by salinity ($F = 94.11$, $n = 4$, $p=<0.0001$; Table 14). Germination was greater at the 0 and 5 psu treatments compared to the 10 and 15 psu treatments (Table 15). Seeds germinated in all treatments with germination increasing over a period of approximately 15 to 20 days depending on the treatment before reaching a plateau (Figure 15). Average germination for the 0 and 5 psu treatments was 75 ± 0.3 percent and 63 ± 0.9 percent respectively. When salinity was increased by 5 psu to the 10 psu treatment germination decreased by 45 percent to 18 ± 0.5 percent. Final germination decreased by half from 18 to 9 percent when seeds were exposed to a salinity of 15 psu (Figure 16).

Mean time to germination (MTG) increased linearly with increasing salinity ($r^2 = 0.93$; Figure 17). The lower salinity treatments, 0 and 5 psu, had a lower MTG of 15 and 20 days compared to 29 and 30 days for the 10 and 15 psu treatments. On average germination of *V. americana* seeds was delayed for a period of two weeks at salinities above 10 psu compared to seeds exposed to 0 and 5 psu treatments (Figure 17). The threshold between 5 and 10 psu is reflected in the decrease in overall final percent germination and increase in MTG of *V. americana* seeds.

Temperature

Overall, germination was significantly influenced by temperature ($F = 52.33$, $n = 4$, $p = 0.0001$; Table 16; Figure 18). Post hoc analysis with Scheffe's test revealed that the 13 °C treatment had significantly less germination compared to the 22, 25, and 29 °C treatments (Table 17). On average, final germination of seeds exposed to 13 °C was 2.5 ± 1.0 percent. A temperature increase of 7 °C to 22 °C increased germination to 75 ± 10 percent. Germination increased with increasing temperature to 86.5 ± 5 percent at 25 °C and 97 ± 2 percent at 29 °C ($r^2 = 0.99$; Figure 19).

Although seeds germinated in all treatments, the small number of germinating seeds in the 13 °C treatment was not sufficient to determine MTG. For the other treatments MTG decreased with increasing temperature (Figure 20). The MTG of seeds exposed to 22 °C was 12 days, seeds exposed to 25 °C MTG decreased to 9 days, and for exposed to 29 °C MTG was the shortest at 6 days (Figure 20). The significant difference in overall germination and MTG between 13 °C and the 22, 25, 29 °C treatments is characteristic of a temperature threshold between 13 and 22 °C that cannot be defined by this data set.

Sediment Type and Burial Depth

Sediment type and burial depth both had a significant effect on germination (sediment type: $F = 35.35$, $n = 5$, $p=0.0001$; burial depth: $F = 4.78$, $n = 4$, $p=0.0010$; Table 18). However, post hoc analysis of sediment type and burial depth with Scheffe's test indicated that only one depth comparison (7 mm x 50 mm) was significantly different

from the other planting depths; therefore, overall burial depth was not considered to have a significant affect on germination of *V. americana* seeds (Table 19).

After data were tested by sediment type only (assuming no depth effect) final germination was found to decrease with decreasing percent NS sediment content ($F = 28.63$, $n = 5$, $p=<0.0001$; Table 20). Germination of seeds in the sediment type treatments was significantly different between the 0 and 25 percent and the 75 and 100 percent NS treatments (Table 21). The 50 percent NS treatment was not significantly different from the 25 or 75 percent (Table 21) but was significantly different than the 0 percent and 100 percent treatments (Table 21, Figure 21).

Final germination was lower in the STBD experiment compared to all other experiments (Figures 22 – 24). The greatest germination percentage (100 percent NS sediment treatment) was only 17 percent compared to 75 percent for the dissolved oxygen and salinity experiments and 97 percent for the temperature experiment (Figures 14, 15, and 18 in which seeds were held in water). In the STBD experiment, germination decreased from 17 ± 2 percent in the 100 percent NS treatment to 13 ± 2 percent in the 75 percent NS treatment. Germination in the 50 percent NS treatment was slightly less than the 75 percent treatment with 8 ± 1 percent. Germination in the 25 and 0 percent NS treatments decreased further to 3 ± 1percent to less than 1 percent respectively (Figure 22).

As percent NS content increased the proportion of sand in the sediment increased and the organic content decreased (Table 9). Based on regression analysis, as the amount of sand in the sediment increased, germination increases exponentially ($r^2 = 0.99$; Figure

23) and when organic content increased, germination decreases exponentially ($r^2 = 0.96$; Figure 24).

Mean time to germination decreased slightly with decreasing mixtures of near shore sediment. Seeds in the 100 percent NS treatment germinated on average in 28 days compared to the seeds in the 0 percent NS treatment that germinated in 32 days. Seeds in the 25, 50, and 75 percent NS treatments germinated on average on days 31, 30, and 29 respectively (Figure 25). Percent sand and percent organic matter had similar effects on time to germination with a linear decrease over time for sand ($r^2 = 0.99$ percent; Figure 26) and an increase over time for percent organic matter ($r^2 = 0.88$ percent organic; Figure 27). Overall MTG for all seeds in the STBD experiment was greater than all other experiments except for the seeds exposed to salinities of 10 and 15 psu.

Criteria Development

Results of all experiments were compared to measurements of their respective factors in Nanjemoy Creek during the month of April when *V. americana* seed germination occurs (Catling et al., 1994). Since the longevity of *V. americana* seeds is currently unknown and a percentage of the seeds were still viable after the completion of all experiments, treatments resulting in lower germination percentages and a larger MTG can only be characterized as delaying germination but not completely inhibiting germination. Therefore when developing the criteria for environmental conditions "optimal" for *V. americana* germination, those conditions which increased overall germination and decreased MTG were considered to be "optimal". Those conditions that

resulted in lower germination or increased mean germination time compared to "optimal" conditions were considered to delay germination.

Water column dissolved oxygen (DO) levels ranged from 9.75-11.21 mg l^{-1} in April 2004 in Nanjemoy Creek. Experimental DO levels for the oxygenated treatment were recorded at 10.70 mg l^{-1}. The oxygenated treatment was considered to be "optimal" compared to the hypoxic experiment (Table 13). Criteria were defined as oxygenated conditions for "optimal" germination and delayed germination under hypoxic conditions.

Salinities ranged between 1.55 – 1.87 psu in Nanjemoy Creek during the month of April (App. 1). These salinities are within the 0 to 5 psu range, which had greater overall germination and shorter germination times than the 10 and 15 psu treatments (Figure 16). Therefore, optimal conditions were defined as ≤5 psu whereas salinities > 5 psu were considered to delay germination (Table 25).

Seeds in temperatures above 22 °C had significantly higher germination and significantly lower mean germination times compared to seeds exposed to 13 °C (Figure 19; Table 17). In April water temperatures in Nanjemoy Creek increased from 11 to 16 °C. The effects of temperatures between 13 and 22 °C on *V. americana* seed germination are not defined by the experimental results; therefore the actual "optimal" temperature that would increase germination of *V. americana* under field conditions cannot be defined (Figure 20). In order to incorporate the range of temperatures between the 13 and 22 °C treatments and water temperatures measured under field conditions, criteria for "optimal" seed germination were defined as >13 °C and temperatures ≤13 °C delayed germination (Table 25).

Nanjemoy Creek sediments varied in percent sand, silt, and clay content but were all defined as either clayey sands or sand (Table 9). Organic content of Nanjemoy Creek sediments was less than 2 percent for all sites in April. In the sediments used for the STBD experiment, as percent NS content in the sediments increased the amount of sand increased (0 percent NS = 3 percent sand; 100 percent NS = 86 percent sand) and the percentage of organic matter decreased (0 percent NS = 8 percent organic matter; 100 percent NS = 1 percent organic matter). Because both factors varied substantially between treatments and can effect SAV production (Barko et al., 1991), these factors were used to define the "optimal" sediment type for *V. americana* seed germination. "Optimal" germination conditions were defined as sediments with < 3 percent organic matter and greater than 40 percent sand content (Table 25). Sediments containing > 3 percent organic matter and < 40 percent sand content were determined to delay *V. americana* seed germination (Figures 23 and 24).

DISCUSSION

Environmental conditions may positively or negatively influence production, reproduction, and restoration of submerged aquatic vegetation (SAV). Germination of *Vallisneria americana* seeds was enhanced (greater overall germination and shorter time to germination) under oxygenated conditions, at temperatures >13 °C, salinities < 5 psu, < 3 percent sediment organic content and > 40 percent sand (Table 25). Light (< 160 µm m^{-2} s^{-1}) and burial depth (down to 10 cm) had no significant effect on germination. In 2004, both production and reproduction of *V. americana* were related to temperature (biomass, density, leaf length increased as temperature increased and flowering occurred when temperatures were the greatest). Production was not markedly affected by any other measured parameter. Based on the synthesis of field and laboratory data, criteria for restoration of *V. americana* using seeds in Chesapeake Bay should include dissolved oxygen, temperature, salinity, and sediment composition. By investigating factors other than water quality, this research provides the initial steps towards quantifying environmental effects on *V. americana* seed germination supplying basic information necessary for the development of restoration criteria for this species.

Temperature

Temperature is an important controlling factor in *V. americana* bed development and seed germination. While the relationships between these factors observed in Nanjemoy Creek may be related to a lack of any seasonal salinity intrusion, the strong correlation between temperature changes and *V. americana* productivity indicate that

seasonal temperature effects on "parent" plants should be included when investigating environmental influences on *V. americana* reproduction.

Vallisneria americana bed development in Nanjemoy Creek during the 2004 growing season was primarily influenced by temperature. Biomass, density, and leaf length of *V. americana* reached maximum values and flowers were produced in July and August after water temperatures increased above ranged from 25 °C for several weeks. Similar trends were observed in *V. americana* beds where production of biomass increased when temperatures increased above 20 °C (Barko et al., 1982).

In laboratory experiments conducted on seeds from Nanjemoy Creek, temperature was also found to play a large role in influencing time to and overall final germination in *V. americana* seeds. An increase of 7 °C from 13 to 22 °C resulted in a 25 – fold increase in germination. In addition a 7 °C increase from 22 to 29 °C resulted in a 50 percent reduction of mean time to germination. Therefore, when other environmental factors are constant, temperature is a strong influence on overall *V. americana* seed germination with a threshold temperature between 13 and 22 °C that, when surpassed, initiates germination. Similar effects of temperature on germination have been reported for other species of freshwater SAV. Hartleb et al. (1993) reports that temperatures of 15 °C are required for germination of *M. spicatum* and Lal and Gopal (1993) report that seeds of *H. verticillata* had exhibited greater overall germination when temperatures increased from 23 to 28 °C.

In April 2004 water temperatures in Nanjemoy Creek were observed to increase 7 °C from 10 to 17 °C. Based on the relationship defined from germination experiments under controlled laboratory conditions, the maximum germination potential of seeds in

the seed bank during this time period would be approximately 40 percent. However, few seedlings were observed in the field during this period, suggesting that other factors were controlling seed germination in these established beds. In reality, germination would probably be less than 40 percent due to the influences and interactions between other confounding factors such as salinity, sediment type, seed viability, and predation.

Salinity

Vallisneria americana production (biomass, density, leaf length, or flowering) during the 2004 growing season did not mirror salinity fluctuations in Nanjemoy Creek. A large pulse of salinity occurred between June and July, which increased salinity from 1.55 to 4.29 psu. Biomass increased at all three sites between May and July with site A experiencing the largest increase from 58.49 ± 10.31 g DW m^{-2} to 314.24 ± 10.03 g DW m^{-2}. Increasing biomass during a period of increased salinity is the opposite of what is expected of freshwater SAV, and indicates that salinity concentrations between 1 and 5 psu did not have a significant negative effect on *V. americana* growth. French and Moore (2003) report that under laboratory conditions, *V. americana* light requirements, necessary for production, increase by as much as 50 percent when salinity is increased from 0 to 5 psu; however, high levels of light (shoots receiving up to 28 percent surface irradiance) can ameliorate the effects of increased salinity. Therefore, it is possible that light levels in Nanjemoy Creek may have lessened any negative effect of salinity and allowed *V. americana* biomass to increase. Therefore, temperature, not salinity, was likely the dominant factor influencing *V. americana* growth and production.

In other estuarine systems where temperature is not as variable as in the Chesapeake Bay, salinity is the main control on *V. americana* growth and survival. For example, transplants along a salinity gradient in the Caloosahatchee Estuary in Southwestern Florida survived in salinities up to 15 psu for at least 6 weeks (Kraemer et al., 1999). However, while surviving under hyper-saline conditions, protein and carbohydrate contents decreased in both above and belowground material. Twilley and Barko (1990) reported that under laboratory conditions, salinities above 2 psu inhibited inflorescence production of *V. americana*, *Potomogeton perfoliatus* and *H. verticillata,* while *M. spicatum* was not effected by salinities up to 12 psu. This suggests that although established *V. americana* shoots may survive at high salinities they are not able to expend as much energy on reproduction whether sexual (above ground) or asexual (below ground) as shoots in lower salinities and therefore may eventually be out competed.

Increased salinity also had a significant negative effect on *V. americana* seed germination (Figure 16). Laboratory results reported here indicate that there may be a threshold effect between 5 and 10 psu that, once surpassed, delays germination. A similar threshold was observed between 5 and 10 psu (for plants receiving 28 percent of surface irradiance) on overall *V. americana* production (including flowering and winter-bud production) during a season long experiment investigating the effects of salinity on *V. americana* (French and Moore, 2003).

In summary, salinity was not a major influence on *V. americana* bed development in Nanjemoy Creek due to salinities never exceeding the tolerance limits of the species and possible amelioration due to light. Salinities between 5 and 10 psu in laboratory

studies did have a significant negative effect on germination, (40 percent reduction in germination) and mean time to germination doubled. Salinity is an important environmental factor that has a large influence on allocation of energy to production, reproduction, or photosynthesis. Depending on the system, salinity can have greater influence on SAV growth and production than temperature (Kraemer, 1999).

Light

Light (< 160 μmol $m^{-2}s^{-1}$) had no effect on seed germination under the controlled laboratory conditions reported here. These results support the conclusions of Muenscher (1936), that exposure of *V. americana* seeds to low levels of diffuse light does not negatively affect germination. Kimber et al. (1995) also concluded that seeds of *V. americana* do not require light to germinate, as seeds germinated in sediments under all light reduction treatments (2, 5, 9, and 25 percent available light). Kimber et al. did find, however, that seedling survival increased significantly under the higher light conditions (9 and 25 percent surface irradiance) compared to those seedlings exposed to low light conditions (2 and 5 percent surface irradiance; Kimber, 1995). This is similar to the results of other studies, specifically French and Moore (2003) who found similar effects on *V. americana* growth from tubers.

Light conditions within SAV beds in Nanjemoy Creek never exceeded the freshwater SAV light habitat criteria of a $K_d < 3.6m^{-1}$ to a depth of 1 m (Batiuk et al., 2000). Although average water column concentrations of both chlorophyll *a* (average 19.85 ± 3.58 μg l^{-1}) and total suspended solids (average 21.11 ± 2.70 mg l^{-1}) exceeded Chesapeake Bay SAV habitat criteria of 15 μg l^{-1} and 15 mg l^{-1} respectively (Batiuk et

al., 2000), *V. americana* beds still received the necessary amount of light to grow and reproduce. Since light levels did not decrease to intensities low enough to significantly limit biomass, density, or flowering, the effects of light limitation on *V. americana* bed development, cannot be quantified here.

A separate study on establishment, growth and survival of *V. americana* transplants in the freshwater region of the James River in Hopewell, Virginia (Moore et al., 2005) reports that *V. americana* shoots have a high tolerance to low light conditions. For example, at one transplant site (Tar Bay) transplants survived under low light levels (K_d = 4.20 m^{-1} and 4.03 m^{-1}) for two continuous growing seasons. In 2004, average water clarity increased and average K_d values were close to the minimum habitat requirements of 3.6 m^{-1}. During the mid-summer all shoots were cropped at approximately 2.5 cm from the basal meristem and died from an apparent lack of light due to herbivory. Grazers reduced the amount of leaf material available for photosynthesis and the majority of shoots died during the summer of 2004. Therefore in the absence of grazers, if the amount of available light fell below the minimum light requirement defined for freshwater SAV in Chesapeake Bay, it is expected that *V. americana* beds would still develop and possibly out-compete other SAV species with higher light requirements.

In summary, while adequate light is a requirement for successful *V. americana* bed development and reproduction, over the 2004 *V. americana* growing season in Nanjemoy Creek it was not limiting. Since moderate (< 160 μmol $m^{-2}s^{-1}$) light conditions were not found to affect germination of *V. americana* seeds, variations in light due to turbidity or depth should not affect germination of non-buried seeds. Research indicates that exposing seeds to long periods of direct light will inhibit germination and may

decrease viability of exposed seeds (Muenscher, 1936). Therefore, when storing seeds it would be important to minimize their exposure to direct light by storing the seeds in a dark place or in a dark container. In addition, since light does effect the survival of *V. americana* seedlings (Kimber et al., 1995), future research investigating the light requirements of seedlings is necessary for restoration purposes.

Dissolved Oxygen

Laboratory analyses reported here of the *V. americana* seed germination was enhanced and mean time to germination decreased in the presence of oxygen. In contrast, hypoxic conditions are known to enhance seed germination in other SAV species. For example, the presence of DO decreases germination in *Z. marina* seeds in Chesapeake Bay, and seeds buried at 5 cm had significantly less germination than seeds buried at 15 and 25 cm (Moore et al., 1993). Additional studies on *Z. marina* seed germination also conclude that hypoxic conditions enhance germination of *Z. marina* from less than 40 percent under oxygenated conditions to greater than 90 percent under hypoxic conditions (Probert and Brenchly, 1999). This suggests varying strategies for submerged aquatics in relation to DO and burial depth.

The effects of water column dissolved oxygen on the development of *V. americana* beds in Nanjemoy Creek cannot be determined due to a lack of hypoxic episodes during sampling. DO concentrations between 6.84 and 11.83 mg l^{-1} were observed over the course of the 2004 growing season in no apparent pattern. Increases in DO at one site did not correspond with increases or decreases at other sampling sites, temperatures, or salinity. In addition, each site was sampled at a different time of day

with site A sampling occurring first, then site B, and finally site C. The time of sampling varied monthly and the effects of different light levels due the time of day on photosynthesis may also affect any possible trends in monthly DO levels.

Redox profiles of sediment cores taken in April from each site show those sediments from within the bed area were oxygenated to depths of 5 cm. Although redox was only measured during April, release of oxygen into the sediment by *V. americana* roots results in the oxidation of sediments (Wigand et al., 1997). As the SAV bed develops and biomass along with shoot density increases, the amount of oxygen released into the sediments may also increase. In a field study in the upper Chesapeake Bay, average redox levels were much greater down to 4 cm depths at + 125 mV in SAV beds dominated by *V. americana* when compared to sediments dominated by *H. verticillata* with mean redox levels of –5 mV (Wigand and Stevenson, 1997). *Vallisneria americana* was the dominant SAV species at the three Nanjemoy Creek sites, therefore it can be concluded that at least the upper 5 cm of the sediment profile were well oxygenated and any plants or seeds within this area were exposed to oxygenated conditions.

Sediment Type

After light, salinity, and temperature, sediment type is a principal factor affecting SAV distribution and growth (Livingston et al., 1998). Sediment from SAV beds sampled in Nanjemoy creek ranged from clayey sands (sites A and B) to pure sand (site C; Shepard, 1954). Although the percent sand silt and clay ratios were measured from cores collected in April, the overall composition of the sediments was not expected to change significantly over the course of one growing season. Therefore, the sediment

classification descriptions were used to describe the sediment at the sampling cites over the entire course of the sampling season and not just for April. In addition, the assumption that percent sand, silt, and clay ratios did not change over the time infers that SAV bed development is not singularly influenced by sediment composition. Characteristics associated with sediment composition, for example the lack of nutrients in soils with high sand content, may change over the course of the season and influence bed development (Barko et al., 1991).

Organic content of the sediments did, however, vary significantly over time for site A with a range of organic content between 1.2 ± 0.10 and 6.9 ± 0.49 percent. In addition to the large change in organic content over a short period of time, there was no difference in organic content in the top 10 cm, suggesting that the source of organic material was well mixed into the top 10 cm over short time periods (less than a month). While, bioturbation of the sediments by benthic organisms can cause a complete reworking and homogenous mixture of the sediments at depths of 5 – 10 m (Barko et al., 1991), the degree of bioturbation in the field sites was not measured.

Such a large increase in organic content could potentially cause a decrease in SAV biomass, density, etc. if a large portion of the accumulated organic material was refractory or was the result of a large accumulation of fine sediments (Barko et al., 1991). A seasonal change in sediment type due to the accumulation of fine sediments appears unlikely in this relatively low energy creek environment. A more likely source of organic material to site A was detritus from SAV present in the bed. After shoot biomass reached maximum levels in July, a large pulse of organic matter to site A sediments occurred.

This increase in the organic content of Nanjemoy Creek sediments may have been related to the overall production of SAV biomass.

Development of *V. americana* biomass in Nanjemoy Creek may have been limited by high levels of sediment organic content. When sediment organic content increased above 5 percent during August in site A, *Vallisneria americana* biomass decreased. Sediment organic content at sites B and C did not increase above 3 percent and biomass production at these sites did not reach maximum levels until the end of the growing season (September). Decreases in biomass production at site A before the end of the growing season supports the findings of Barko et al., (1991) that increased sediment organic content may inhibit SAV growth and production.

While increasing organic matter does not always indicate an increase in nutrients (Barko et al., 1991), August was also the time of greatest increase in sediment porewater nutrients. Increases in ammonia concentration were also observed between July and August. The levels were typical of ammonium concentrations others have found in Potomac River sediments in macrophyte beds (Carter et al., 1987) and are not considered to be a significant factor on bed development. Sediment phosphate levels (PO_4^{-3}) may illustrate the connection between sediment characteristics and the September peak in biomass as SAV beds in freshwater areas are usually P limited not N limited (Barko et al., 1991).

Release of oxygen by *V. americana* roots results in oxidation of sediments, which increases the redox potential in sediment and the oxidation of iron and manganese. These conditions, in turn, can result in the release of phosphorous (Wigand et al., 1997) that the plants can readily absorb. In a study of the influences of *V. americana* on sediment

biogeochemistry of SAV beds in upper Chesapeake Bay, phosphate levels were measured at 1.01 ± 0.07 μM in 1990 and 0.12 ± 0.01 μM in 1995 in beds dominated by *V. americana* (Wigand et al., 1997). These concentrations were 6-50 times lower than concentrations they measured from beds within mixed stands of *H. verticillata* and *M. spicatum* (6.52 ± 0.98 μM). Concentrations of phosphorus measured in Nanjemoy Creek were within the *V. americana* bed levels reported by Wigand et al. (1997).

Based on the sediment characterization, change in organic content, and nutrient levels, the most likely influence of sediment type on bed development of *V. americana* is phosphorous availability. SAV species receive the majority of their nutrients from the sediment (Barko and Smart, 1981). The low nutrient content of the sediments selects for a species that can withstand these conditions. Although the concentrations of NO_x and ammonium were likely not limiting, phosphorus concentrations in the sediment remained below 1 μM for the majority of the growing season. When compared to *H. verticillata*, *V. americana* has been found to be able to out compete the non-native species when sediments were nutrient limited (Van et al., 1999). When N and P were added to the sediment *H. verticillata* quickly grew and became dominant. Therefore, next to temperature, sediment nutrient concentrations in Nanjemoy Creek sediments likely were the most influential environmental factor controlling *V. americana* bed development.

Experimental results reported here demonstrate that *V. americana* seeds had greater germination rates in sand (100 percent NS) or clayey sand (75 percent NS) with an organic content less than 3 percent compared to silty clay (0 percent NS) or mixed (25 percent) sediments with an organic content greater than 3 percent. One explanation for this difference is the relationship between porewater sediment phosphorus content and

sediment composition. When sediment porewater analyses were compared between treatments, phosphorus concentrations were significantly greater in the 100 percent NS treatment compared to all other treatments. Nitrogen was not significantly different between treatments, and since phosphorus can be limiting for freshwater SAV (Barko et al., 1991), greater germination in the 100 percent NS treatment could be attributed to the difference in sediment phosphorus.

An alternate hypothesis for the difference in germination between treatments is the composition of the experimental sediments. Sediment organic content and composition are two environmental factors known to limit SAV growth (Barko and Smart, 1986; Livingston et al., 1998). For example, two freshwater SAV species often found in mixed beds with *V. americana*, *H. verticillata* and *M. spicatum,* grew poorly in sediments with > 75 percent sand and < 10 percent organic matter compared to plants grown in sediments with greater concentrations of organic matter (up to 20 percent; Barko and Smart, 1986). Larger grains, such as sand, increased sediment density and were inversely correlated with sediment nutrient content. Therefore, sediment composition (based on percent sand, silt, clay ratios) inhibited growth more than organic content via nutrient regulation. While nutrient concentrations have been shown to effect overall plant growth, the effects on seed germination are unknown and warrant further research.

Vallisneria americana seed germination reported here was greater under sandy low organic content sediment conditions. In addition, nutrient concentrations were not inversely proportional to sediment sand content as expected (Barko and Smart, 1986). Seeds in treatments with sandier soils were not nutrient limited compared to seeds in the

other treatments; therefore, nutritional limitation due to sediment composition cannot explain the difference between treatments. Germination of *V. americana* seeds under oxygenated conditions resulted in significantly higher rates and lower mean time to germination compared to hypoxic conditions. If sediment treatments with higher organic content (25 percent NS and 0 percent NS) showed reduced oxygen levels, then the difference between treatments may have been due to nutrient limitation from low dissolved oxygen levels. However, redox profiles of the entire sediment column (2 – 10 cm) for each core did not show a significant difference in oxygen potential between treatments or depths. There was a slight difference in redox potential between the sediment water column interface (200 – 350 mV) and 2 mm depths (100 – 200 mV); however sediment conditions were not severely reduced and the majority of the sediment profile remained oxygenated.

Burial depth up to 10 cm was also not a significant factor affecting germination of *V. americana* seeds between treatments in this study. These results support a similar experiment investigating the effects of burial depth on *V. americana* winter-bud survival and germination (Rybicki and Carter, 1986). Survival of *V. americana* winter-buds at depths greater than 25 cm in their study was significantly less than those in ≤10 cm of sediment (< 10 cm = 90 percent survival; > 10 cm = 0 percent survival). Assuming high rates of bioturbation at the Nanjemoy Creek field sites, if seeds are mixed below the 10 cm threshold, the potential for *V. americana* seed germination may decrease significantly and serve as an environmental control on seed germination. Otherwise, all viable seeds within the top 10 cm would comprise the seed bank.

In summary, sediment sand, silt, clay ratios, organic content, nutrient concentrations, burial depths and oxygen levels within the sediments were not able to explain the differences in germination between high sand low organic content and low sand high organic content sediments. In addition, these parameters could not fully explain the difference in maximum germination observed when seeds were planted in sediments (20 percent) compared to seeds observed in water only (63 to 97 percent). Alternate hypotheses for germination differences between the treatments and experiments include varying mechanical resistance of the sediment (i.e. hardened particles stuck together with water) inhibiting germination (Hornbaker et al., 1997), bacterial growth within the sediment, or a lack of mycorrhizae development necessary for nutrient uptake and growth necessary in sediments (Wigand and Stevenson, 1997).

Seed Production and Viability

The calculated amount of seeds produced in Nanjemoy Creek during 2003 was considerably greater than the number of seeds found in the sediment seed bank (10 cm depth) 12 months later (Table 6). Possible reasons for this decrease include seed germination, grazing, burial below seed bank, export of seed pods due to tidal movement, and decay or loss of viability.

Seed Production

Vallisneria americana beds in Nanjemoy Creek did not experience poor water quality conditions and had only one major loss of biomass due to grazers during the 2004 season. The data collected from these sites therefore can be considered an example of *V.*

americana growth under favorable conditions. In Nanjemoy, flowers were produced by 30 – 40 percent of shoots. *V. americana* populations located in the Great Lakes along the Lake Huron-Eerie corridor showed similar flower production with 28 to 60 percent shoots flowering (Lokker et al., 1997).

Vallisneria americana is dioecious, therefore the ratio of male to female flowers produced influences the number of successfully pollinated flowers. For *V. americana* sex ratio can be male or female biased depending on location (Lokker et al., 1997). Due to the release of male flowers from the basal meristem prior to sampling in August, the sex ratio in Nanjemoy Creek could not be determined and for purposes of discussion is assumed to be equal. Assuming that 50 percent of the flowers produced in 2004 were female and that all female flowers are successfully pollinated, then 71 ± 4 seed pods m^{-2} were produced at site A, 110 ± 5 seed pods m^{-2} were produced at site B, and 30 ± 11 seed pods m^{-2} were produced at site C. Approximately 2,000 seed pods were collected from a 5 m^{-2} area in one *V. americana* bed in Nanjemoy Creek during October 2003 and again in October 2004. Therefore, while the numbers presented represent an upward bound of seed production in Nanjemoy Creek, they are not completely unrealistic.

On average, *V. americana* seed pods have been found to contain between 100 and 300 seeds (Looker et al., 1997). In Nanjemoy the mean number of seeds produced per seed pod was 150 seeds. Therefore, the calculated number of seeds produced during the 2004 growing season in Nanjemoy Creek ranged from 4,500 to 16,000 seeds m^{-2} depending on sampling site. For other species of SAV mean density of seed production is significantly less than the numbers presented here. For example, the average number of Z. *capricorni* seeds in the sediment of Morton Bay, Australia was 2,904 ± 149 seeds

m^{-2} (Conacher et al., 1994) and mean seed production of *Z. marina* in Chesapeake Bay has been estimated at estimated at 8,127 seeds m^{-2} (Silberhorn et al., 1983). The main difference in seed production between these species is the volume of seeds produced. One *V. americana* seed pod can produce up to 300 individual seeds whereas some species of SAV produce single seeds up to tens of seeds.

Vallisneria americana seeds produced in the Lake Huron-Erie corridor were counted and compared to the number of seeds found in the seed bank. Ten times more seeds were produced than were found in the established seed reserve (Looker et al., 1997). Various factors can account for the difference in seed production compared to seed bank reserves including germination, predation, dispersal, and environmental conditions (Westcott et al., 1997). When *V. americana* plants are light or salinity stressed production of winter-buds and or flowers decreases (French and Moore, 2003). If plants are stressed during the time of seed production, then overall production of seeds may decrease; subsequently the flux of seeds to the seed bank may also decrease. Due to habitat differences, germination, grazing, dispersal, changing environmental conditions, and a lack of distinction between seeds produced in different years, it is hard to accurately calculate the number of seeds produced based on the seed bank reserves.

However, based on water quality data from the Chesapeake Bay Program, water quality conditions in Nanjemoy Creek were similar between 2003 and 2004. Therefore, environmental conditions should be approximately equal for both years. Assuming dispersal, germination, and predation were similar between the two seasons, the calculated number of seeds produced in 2003 is assumed to be equivalent to the observed number of seeds produced in 2004. Given this, seeds collected from sediment cores in

Nanjemoy Creek indicate that after a period of 12 months <1 percent of seeds produced remained in the sediment. The small number of *V. americana* seeds deposited in the seed bank reported here, 590 to 1,100 seeds m^{-2}, was similar to the number of seeds in *V. americana* beds in the Great Lakes where the number of seeds deposited ranged from 73.6 ± 22.9 to 878.2 ± 268.6 seeds m^{-2} (Lokker et al., 1997). Lower estimates of *V. americana* seed production are given for Presqu'ile Bay in Canada where 0.2 ± 0.2 seeds were found between 0-14 cm on average (Westcott et al., 1997). In addition, few seedlings were observed in the field here, therefore, the difference in the number of *V. americana* seeds produced and the number of seeds observed in the seed bank was not primarily due to germination. Another removal mechanism such as dispersal, deep burial, or predation could possibly have a large influence on the size of the seed bank.

Export and predation may play a large roll in the dramatic difference between *V. americana* seed production and seed bank reserves. Seed pods contain a gelatinous mass surrounding the seeds that serves as a potential food source for migrating waterfowl (Perry, 1988). Waterfowl can be an effective dispersal mechanism for small hard seeds with a protective coat such as *V. americana* seeds (Figureola and Green, 2002). For every one seed pod that is removed by grazing several hundred seeds are also removed. In addition seed pods are positively buoyant until they begin to decompose (Korschgen and Green, 1988; Titus and Hoover, 1991; Lokker et al., 1997). If a seed pod is still intact after the plant material dies back, seed pods could disperse away from the parent bed, again removing hundreds of seeds from the parent bed and therefore from the seed bank reserve.

Seed Viability

Environmental conditions are hypothesized to significantly influence the ability of a seed to germinate by initiating or inhibiting (delaying) germination. If environmental factors such as salinity, temperature, or sediment composition are the main influence on the ability of a seed to germinate, then one logical reason for seeds to not germinate under the "optimal" conditions would be a lack of viability. If the environmental conditions tested here by each experiment were responsible for the presence or absence of germination in *V. americana* seeds, then the number of non-viable seeds remaining at the conclusion of the experiment should decrease with increasing germination.

The optimal temperature for seed development was between 22 and 29 °C with germination increasing with temperature. Viability of non-germinating seeds decreased with increasing temperature. Seeds in the 29 °C treatment had the greatest amount of germination (97 percent) and the lowest viability in the remaining seeds (25 percent) whereas the 13 °C treatment had only 3 percent germination and 89 percent of the remaining seeds were viable. In the temperature experiment, cooler temperatures did not cause the development of non-viability in the majority of *V. americana* seeds. Therefore if no other environmental factors are limiting, an increase in temperature will initiate germination and the number of viable seeds remaining in the sediment will decrease (Table 22).

Salinities in Nanjemoy Creek never increased over 5 psu during the 2004 growing season. The results of the salinity experiment indicate that approximately 75 percent of *V. americana* seeds produced will germinate when salinities are below 5 psu. In addition 21 to 31 percent of the remaining seeds, or 16-35 seeds m^{-2} will be viable after 12 months

(Table 23). Although germination decreases, viability remains at approximately 21 percent when salinities increase to 10 and 15 psu. Therefore, salinities > 5 psu do not inhibit germination by reducing seed viability. This suggests that higher salinities could delay germination until salinity concentrations drop to < 5 psu and, therefore, increase the chances of successful seedling establishment.

During the sediment type burial depth experiment, maximum germination occurred in sediments with < 2 percent organic matter and > 75 percent sand content. However, viability testing revealed that viability of remaining seeds did not have a direct relationship to sediment type (Table 23). On average 60 percent of the remaining seeds were viable independent of what treatment they experienced. Viability testing further indicates that an additional factor separate from sediment composition (percent sand silt clay and organic content) might possibly affect seed germination. However, based on the synthesis of data from the sediment experiment and the observed sediment conditions in Nanjemoy Creek, approximately 60 percent of seeds remaining in the sediment after 12 months will still be viable. Given estimates of seed production here this would equal 172 seeds m^{-2} (Table 24). The number of viable seeds estimated from the sediment experiments is substantially greater when compared to the number of viable seeds observed at all three sites in Nanjemoy Creek. This suggests other factors beyond sediment effects are responsible for the decrease in seed viability in the Nanjemoy seed bank.

Nanjemoy Creek water temperatures as high as 31 °C and salinity levels were not recorded above 5 psu during the 2004 growing season. Based on the results of the temperature and salinity experiments, the number of viable seeds remaining in the

sediments after 12 months would be approximately 2 seeds m^{-2} and 16 – 35 seeds m^{-2} respectively. The number of expected viable seeds based on the sediment type burial depth experiment increases to 172 seeds m^{-2}. In comparison, 1 percent of seeds collected in Nanjemoy Creek in 2004 were viable leaving 36 seeds m^{-2} at site A and 83 seeds m^{-2} at site B with none at site C. Results from all experiments here are within the range of the number of observed viable seeds. Therefore, the relatively low number of observed seeds in Nanjemoy Creek, compared to the number of seeds produced, could possibly be explained by the influences of environmental conditions on seed germination.

CONCLUSIONS

Environmental conditions have significant effects on *V. americana* seed germination and bed development. Once a temperature threshold of 13 °C is surpassed, germination is initiated, and at 25 °C biomass production increases markedly. Despite some halo-tolerance, *V. americana* is a freshwater plant and, as such, salinities ≤ 5 psu enhance germination and overall bed development. Salinities ≥ 10 psu delay germination, but do not effect seed viability. This may allow germination to continue once salinity decreases and conditions are more conducive for plant survival. Germination is enhanced in oxygenated sandy substrates compared to clay or silty substrates. However, germination rates are dramatically lower in seeds in water treatments compared to those in sediments. The overall effect of substrate on germination was not resolved. Sediment composition and organic content explain only a portion of the story and further research into areas such as bacteria populations, mycorrhizae availability, and the effects of bioturbation on seed germination need to be investigated.

While some environmental factors have more control on germination than others, the interactions between all environmental conditions need to be further understood before the effects of any environmental factor can be negated. For example, light and burial depth did not have a significant effect on seed germination when investigated singularly. However, the effects of burial depth and additional environmental parameters such as temperature, light, or salinity were not quantified and should be considered in any future research on *V. americana* seed germination. In addition, not all variability in seed

production and germination may be attributable to the parameters investigated here. For example, thousands of seeds were produced in Nanjemoy Creek and only tens of viable seeds were found in the seed bank. Reasons for this discrepancy are unknown but may include lose of seeds due to grazing of seed pods, dispersal of seeds or seed pods through currents, or decomposition of seeds in the sediment. Reasons for the small number of seeds found in the seed bank need to be further understood to help identify the importance of seeds in *V. americana* ecology. Do seed pods or seeds serve as dispersal mechanisms? Can they remain viable after consumption?

The environmental conditions described by the criteria developed for *V. americana* seed germination were based on laboratory results and observations from Nanjemoy Creek during 2004. It is important to note that these criteria were developed partially based on a single growing season in one creek. Variability of environmental conditions occur on many scales, including interannually and spatially, and observations of environmental conditions over several additional years in various locations would strengthen the criteria developed here.

These criteria are a first step to quantifying the environmental conditions that enhance and or delay germination of *V. americana* seeds. As restoration of SAV progresses from transplanting whole shoots to methods utilizing seeds, investigations into the role of environmental factors on all aspects of sexual reproduction (flower production, seed development, seed germination, seedling establishment, and seedling survival) need to be thoroughly researched. Continuation and expansion of research on sexual reproduction can provide basic knowledge missing for many SAV species and increase the effectiveness of SAV restoration.

Table 1. Mean values of Nanjemoy Creek sediment organic content (percent organic) ± SE. Sediment classification based on percent sand, silt, and clay ratios (Shepard, 1954). Percent sand silt clay ratios of sediments ± SE.

	Site A	Site B	Site C
% Organic			
April	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.2
June	4.2 ± 0.4	2.3 ± 0.6	1.1 ± 0.2
July	4.4 ± 0.3	2.0 ± 0.1	1.7 ± 0.6
August	6.9 ± 0.5	2.5 ± 0.3	1.9 ± 0.5
September	5.4 ± 0.2	1.5 ± 0.1	1.3 ± 0.2
October	2.2 ± 0.3	1.4 ± 0.2	1.42 ± 0.2
Sed. Classification	Clayey Sand	Clayey Sand	Sand
% Sand, Silt, Clay			
% Sand	50 ± 5	67 ± 1	88 ± 1
% Silt	13 ± 2	12 ± 2	2 ± 0.4
% Clay	37 ± 5	21 ± 3	10 ± 1

Table 2. Mean biomass of *V. americana* shoots in the mid portion of SAV beds (40 – 80 m for sites A and B; 20 – 30 m site C) from Nanjemoy Creek in 2004. Weights reported in g dry weight m^{-2} ± SE. No biomass samples were collected from sites B and C in June.

Month	Site A	Site B	Site C
April	39.79 ± 6.64	11.46 ± 1.90	1.36 ± 0.34
May	58.49 ± 10.31	56.32 ± 11.97	16.03 ± 2.25
June	145.92 ± 13.58	.	.
July	314.24 ± 10.03	195.31 ± 15.62	107.43 ± 9.79
Aug	290.54 ± 15.84	327.09 ± 9.41	117.44 ± 12.39
Sept	287.85 ± 45.76	377.48 ± 25.70	296.32 ± 39.13
Oct	231.68 ± 11.44	60.59 ± 8.97	126.59 ± 14.53

Table 3. Mean percent cover of *V. americana* shoots in the mid portion of SAV beds (40 – 80 m for sites A and B; 20 – 30 m site C) from Nanjemoy Creek in 2004. Percent cover reported in percent cover m^{-2} ± SE. Data on percent cover from mid-portion of SAV beds was not collected in April or May.

Date	Site A	Site B	Site C
June	4 ± 2	18 ± 11	13 ± 9
July	94 ± 5	94 ± 5	39 ± 23
August	92 ± 2	91 ± 1	40 ± 23
September	79 ± 8	94 ± 5	46 ± 23
October	84 ± 5	76 ± 15	30 ± 20

Table 4. Mean density of *V. americana* shoots in the mid portion of SAV beds (40 – 80 m for sites A and B; 20 – 30 m site C) from Nanjemoy Creek in 2004. Density is reported as # shoots m^{-2} ± SE. Data on shoot density was not collected in April, May, September, or October due to weather conditions.

Date	Site A	Site B	Site C
June	53 ± 17	78 ± 23	21 ± 11
July	200 ± 14	240 ± 13	95 ± 43
August	230 ± 12	270 ± 10	110 ± 29

Table 5. Mean leaf length of *V. americana* shoots in the mid portion of SAV beds (40 – 80 m for sites A and B; 20 – 30 m site C) from Nanjemoy Creek in 2004. Leaf length is reported in length cm ± SE.

Month	Site A	Site B	Site C
June	37.0 ± 17.6	10.6 ± 5.3	4.7 ± 1.7
July	105.1 ± 5.1	79.5 ± 12.3	7.5 ± 6.8
Aug	133.0 ± 4.7	108.4 ± 15.7	22.2 ± 10.2
Sept	106.8 ± 4.7	88.3 ± 8.4	30.7
Oct	86.1 ± 5.6	55.1 ± 4.6	26.3

Table 6. Mean sexual reproductive output for *V. americana* shoots in Nanjemoy Creek in 2004 based on observations of shoot density, flowering shoot density, number of pods produced and number of seeds per pod from the September 2004 transect data. The number of seeds m^{-2} in the sediment after 12 months and the number of viable seeds m^{-2} in the sediment after 12 months for all sites are counts from the September sediment cores. Numbers are values ± SE.

	Site A	Site B	Site C
# of plants m^{-2}	220 ± 12	260 ± 11	74 ± 35
% flowering shoots m^{-2}	33 ± 22	43 ± 30	30 ± 17
# of pods m^{-2}	71 ± 4	110 ± 5	22 ± 11
# of seeds produced m^{-2}	11,000 ± 590	16,000 ± 730	4,500 ± 1600
# seeds m^{-2} after 12 mo	590	1100	0
# viable seeds m^{-2} after 12 mo	118	230	0

Table 7. Mean temperatures (°C) for all treatments during temperature experiment. Values are reported at means ± SE.

Treatment	Temp (°C)
13	13.0 ± 0.6
22	22.2 ± 0.6
25	24.5 ± 0.5
29	28.6 ± 0.7

Table 8. Mean salinity (psu) for all treatments during salinity experiment. Values are reported at means ± SE.

Treatment	Salinity (psu)
0	0.13 ± 0.02
5	5.24 ± 0.09
10	10.50 ± 0.10
15	15.49 ± 0.17

Table 9. Characterization of sediment used in sediment type and burial depth experiment. Treatments determined by percent NS (near shore) content. Values are reported as means ± SE. Sediment classification index values calculated with percent sand silt clay ratios (Shepard, 1954).

% NS Sediment	0 %	25 %	50 %	75 %	100 %
% Organic	8.3 ± 0.2	4.8 ± 0.0	2.8 ± 0.0	1.7 ± 0.0	0.9 ± 0.1
Sand:Silt:Clay					
% Sand	3 ± 0	38 ± 2	63 ± 0	78 ± 1	86 ± 0
% Silt	37 ± 3	22 ± 2	12 ± 0	6 ± 3	4 ± 0
% Clay	60 ± 2	40 ± 4	25 ± 1	17 ± 2	9 ± 1
Sed. Classification	Silty Clay	Mixed Sediments	Clayey Sand	Clayey Sand	Sand
Nutrients					
$NO_2^- + NO_3^-$ µM	< 0.01 ± 0.0	sample lost	< 0.01 ± 0.0	< 0.01 ± 0.0	< 0.01 ± 0.0
NH_3^+ µM	384.1 ± 19.9	sample lost	370.8 ± 25.7	379.8 ± 125.5	269.5 ± 90.1
PO_4^{-3} µM	0.5 ± 0.1	sample lost	0.4 ± 0.0	0.6 ± 0.0	2.3 ± 0.7

Table 10. Mean percentage of viable non-germinated *V. americana* seeds remaining after completion of the salinity experiment. Values are reported as mean ± SE.

Salinity (psu)	# germ	# non-germ	% viable non germ seeds
0	38 ± 1	12 ± 1	21 ± 8
5	32 ± 3	19 ± 3	31 ± 8
10	9 ± 1	41 ± 1	54 ± 4
15	4 ± 1	46 ± 1	53 ± 6

Table 11. Mean percentage of viable non-germinated *V. americana* seeds remaining after completion of the temperature experiment. Values are reported as mean ± SE.

Temp (°C)	# germ	# non-germ	% viable non germ seeds
13	1 ± 1	49 ± 1	90 ± 1
22	38 ± 6	13 ± 6	77 ± 6
25	43 ± 3	7 ± 3	21 ± 12
29	49 ± 1	2 ± 1	13 ± 13

Table 12. Mean percentage of viable non-germinated *V. americana* seeds remaining after completion of the sediment type burial depth experiment. Values are mean ± SE.

% NS	# germ	# non-germ	% viable non germ seeds
100	9 ± 1	41 ± 1	65 ± 4
75	7 ± 1	43 ± 1	52 ± 6
50	4 ± 1	46 ± 1	56 ± 6
25	2 ± 0	48 ± 0	64 ± 5
0	0 ± 0	50 ± 0	65 ± 4

Table 13. Two-way ANOVA on effects of light and oxygen on germination of *V. americana* seeds. Numbers in bold are significant; n = 2 for both light and oxygen treatments (LD = light/dark and AA = oxygenated/hypoxic).

DO and Light	**DF**	**F**	**p**
Light/Dark	1	0.00	0.9676
Oxygenated/Hypoxic	1	8.72	**0.0094**
LD*AA	1	0.47	0.5015
Residual	16		

Table 14. One-way ANOVA on effects of salinity on germination of *V. americana* seeds. Numbers in bold are significant and n = 4 for treatment.

Salinity	**DF**	**F**	**p**
Treatment	3	94.11	**<0.0001**
Residual	12		

Table 15. Post-hoc analysis of germination data with Scheffe's Test for effects of individual salinity treatments on germination of *V. americana* seeds. Numbers in bold are significant; significance level was five percent.

Treatments	**Mean Diff**	**Crit. Diff.**	**p**
0, 5	0.13	0.17	0.1587
0, 10	0.62	0.17	**<0.0001**
0, 15	0.76	0.17	**<0.0001**
5, 10	0.48	0.17	**<0.0001**
5, 15	0.62	0.17	**<0.0001**
10, 15	0.13	0.17	0.1601

Table 16. One-way ANOVA on effects of temperature on germination of *V. americana* seeds. Numbers in bold are significant; n = 4 for treatment.

Temperature	DF	F	p
Treatment	3	52.33	**<0.0001**
Residual	12		

Table 17. Post-hoc analysis of germination data with Scheffe's Test for effects of individual temperature treatments on germination of *V. americana* seeds. Numbers in bold are significant; significance level was five percent.

Treatments	Mean Diff	Crit Diff.	p
13, 22	-0.89	0.34	**<0.0001**
13, 25	-1.03	0.34	**<0.0001**
13, 29	-1.23	0.34	**<0.0001**
22, 25	-0.14	0.34	0.6503
22, 29	-0.33	0.34	0.0576
25, 29	-0.20	0.34	0.3722

Table 18. Two-way ANOVA on effects of sediment type and burial depth on germination of *V. americana* seeds. Numbers in bold are significant; n = 4 for depth, n = 5 for sediment type.

	DF	F	p
Depth	5	4.78	**0.0010**
Sed Type	4	35.35	**<0.0001**
Depth*Sed Type	20	1.05	**0.4195**
Residual	60		

Table 19. Post-hoc analysis of germination data with Scheffe's Test on effects of burial depth on germination of *V. americana* seeds. Numbers in bold are significant; significance level was five percent.

Treatments	Mean Diff	Crit Diff.	p
2, 7	-0.04	0.18	0.9867
2, 15	0.03	0.18	0.9967
2, 25	0.12	0.18	0.4737
2, 50	0.16	0.18	0.1272
2, 100	0.14	0.18	0.2378
7, 15	0.07	0.18	0.8642
7, 25	0.16	0.18	0.1442
7, 50	0.20	0.18	**0.0221**
7, 100	0.19	0.18	**0.0513**
15, 25	0.08	0.18	0.7835
15, 50	0.13	0.18	0.3366
15, 100	0.11	0.18	0.5202
25, 50	0.05	0.18	0.9808
25, 100	0.03	0.18	0.9987
50, 100	-0.20	0.18	0.99997

Table 20. One-way ANOVA on effects of sediment type only on germination of *V. americana* seeds. Numbers in bold are significant; n= 5 for sediment type.

STBD	DF	F	p
Sed Type	4	28.63	**<0.0001**
Residual	85		

Table. 21. Post-hoc analysis of germination data with Scheffe's Test on effects of sediment composition (percent NS) on germination of *V. americana* seeds. Numbers in bold are significant; significance level was five percent.

% NS	Mean Diff	Crit Diff.	p
0, 25	-0.12	0.17	0.3091
0, 50	-0.28	0.17	**0.0001**
0, 25	-0.41	0.17	**<0.0001**
0, 100	-0.51	0.17	**<0.0001**
25, 50	-0.16	0.17	0.0891
25, 75	-0.29	0.17	**<0.0001**
25, 100	-0.39	0.17	**<0.0001**
50, 75	-0.13	0.17	0.2197
50, 100	-0.23	0.17	**0.003**
75, 100	-0.10	0.17	0.5499

Table 22. Predicted number of *V. americana* seeds m^{-2} in Nanjemoy Creek seed bank based on results of temperature experiment.

Temp °C	# germ m^{-2}	# non germ m^{-2}	# viable non-germ seeds m^{-2}	# viable seeds m^{-2} after 12 mo.
13	220	8590	7620	533
22	6608	2203	1574	110
25	7621	1189	385	27
29	8546	264	66	5

Table 23. Predicted number of *V. americana* seeds m^{-2} in Nanjemoy Creek seed bank based on results of salinity experiment.

Salinity psu	# germ m^{-2}	# non-germ m^{-2}	# viable non-germ seeds m^{-2}	# viable seeds m^{-2} after 12 mo.
0	6652	2158	454	32
5	5550	3260	1000	70
10	1542	7268	3893	273
15	749	8061	4297	301

Table 24. Predicted number of *V. americana* seeds m^{-2} in Nanjemoy Creek seed bank based on results of sediment type experiment.

% NS	# germ m^{-2}	# non-germ m^{-2}	# viable non-germ seeds m^{-2}	# viable seeds m^{-2} after 12 mo.
100	1507	7303	4755	333
75	1184	7626	3975	278
50	685	8125	4515	316
25	284	8526	5491	384
0	29	8781	5672	397

Table 25. Germination criteria for *V. americana* based on synthesis of laboratory data and field observations.

Environmental Parameter	Optimal	Delayed
Temperature (°C)	< 13	> 13
Salinity (psu)	≤ 5	≥ 10
Sediment Composition (% sand)	≥ 40	< 40
Sediment Organic Content (%)	≤ 3	> 3

Figure 1. Map of total SAV coverage for 2003 in Chesapeake Bay (Orth et al., 2004).

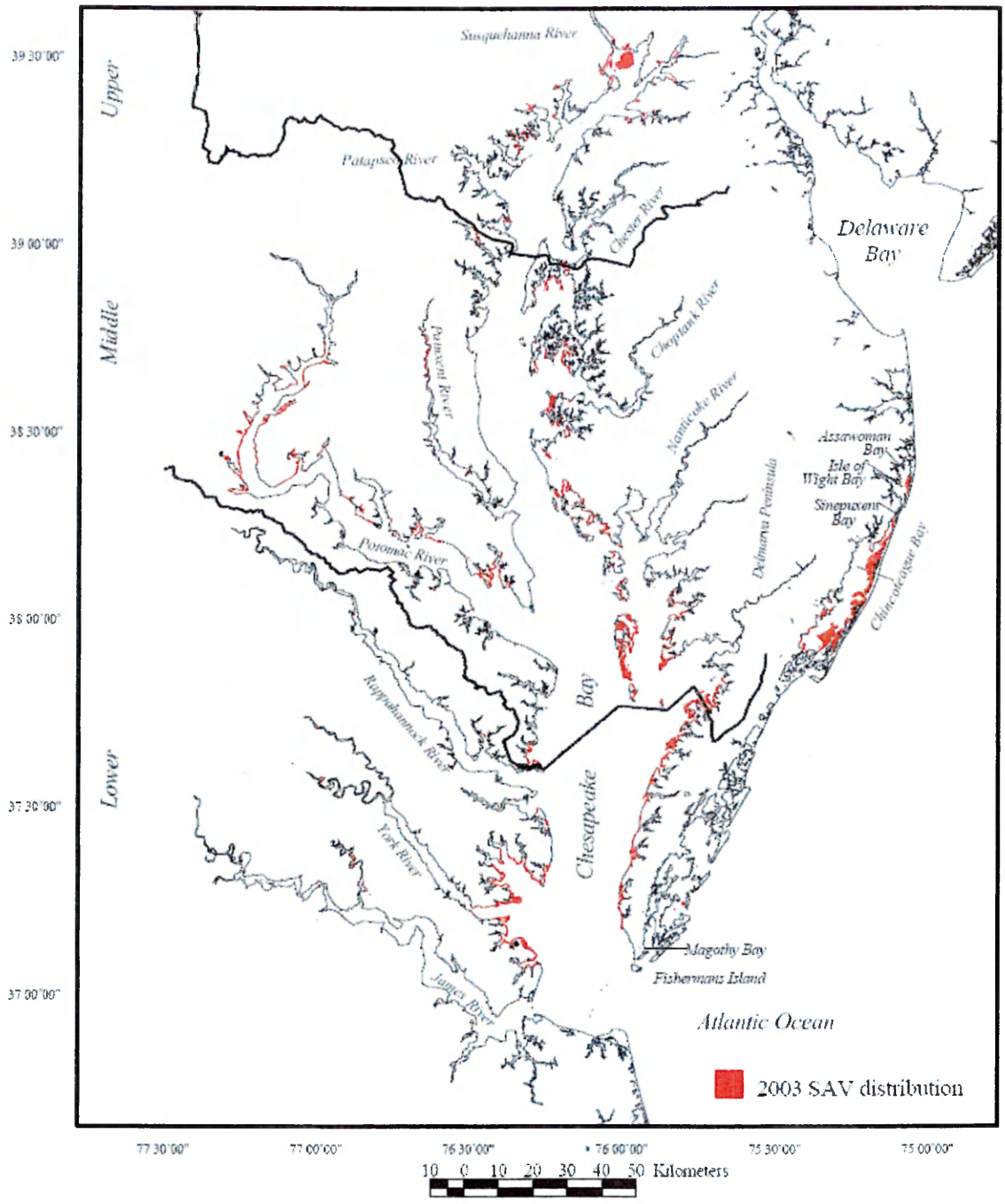

Susquehanna River
Upper
Patapsco River
Chester River
Delaware Bay
Middle
Patuxent River
Choptank River
Nanticoke River
Assawoman Bay
Isle of Wight Bay
Sinepuxent Bay
Delmarva Peninsula
Potomac River
Chincoteague Bay
Rappahannock River
Bay
Lower
Chesapeake
York River
Magothy Bay
Fishermans Island
James River
Atlantic Ocean
2003 SAV distribution
39 30'00"
39 00'00"
38 30'00"
38 00'00"
37 30'00"
37 00'00"
77 30'00"
77 00'00"
76 30'00"
76 00'00"
75 30'00"
75 00'00"
10 0 10 20 30 40 50 Kilometers

Figure 2. Diagram of processes leading to the production and fate of SAV propagules (modified from Titus and Hoover, 1991).

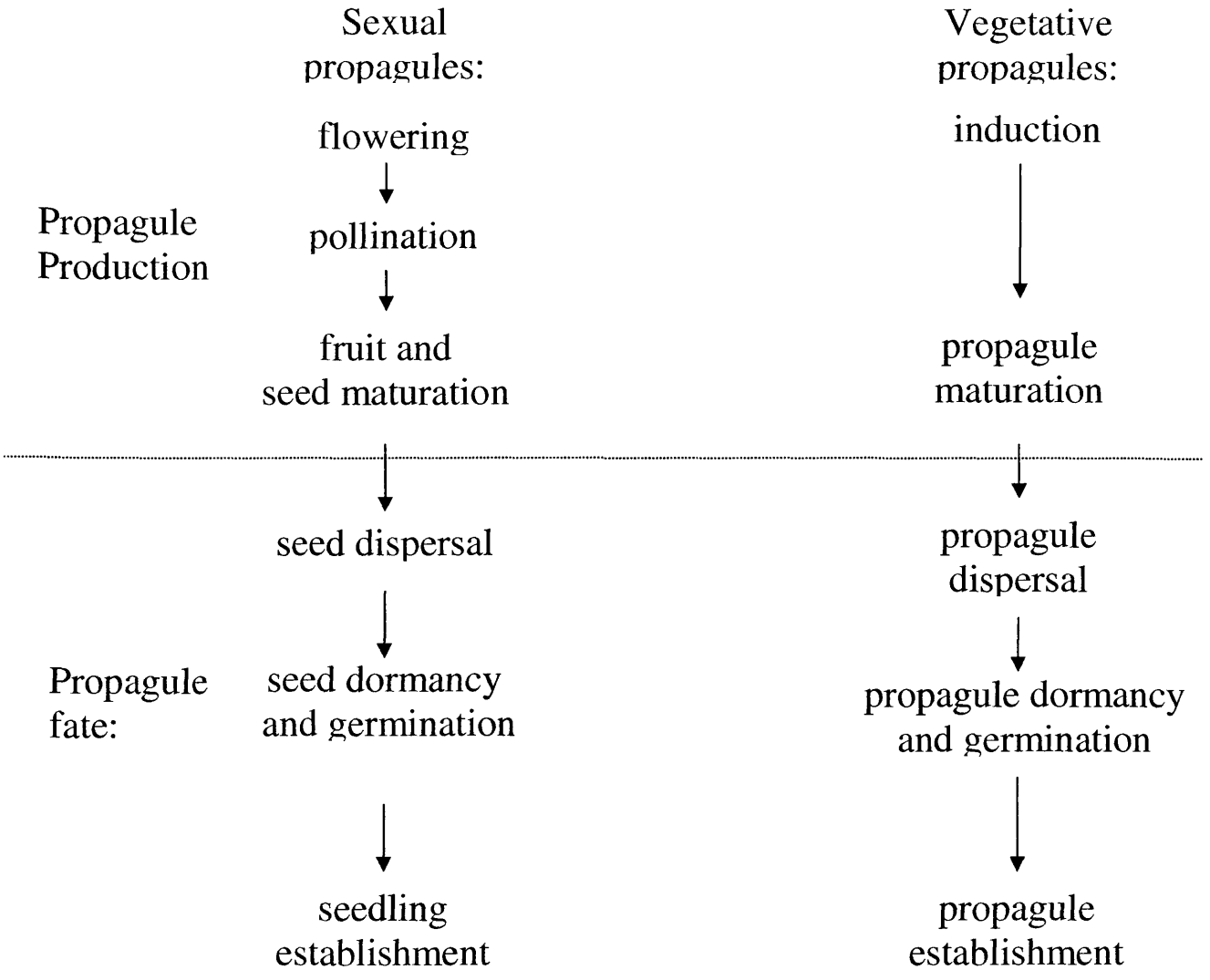

Sexual propagules:
Vegetative propagules:
Propagule Production
flowering
pollination
fruit and seed maturation
induction
propagule maturation
Propagule fate:
seed dispersal
seed dormancy and germination
seedling establishment
propagule dispersal
propagule dormancy and germination
propagule establishment

Figure 3. Location of *V. americana* sampling sites in Nanjemoy Creek, MD. Red highlighted areas are SAV beds in Chesapeake Bay 2003 (Orth et al., 2004). Green shaded areas denote SAV coverage in Nanjemoy Creek in 2002 (Orth et al., 2003). Numbers indicate 2004 sampling sites Site A is the location of seed pod collection during 2003 and 2004.

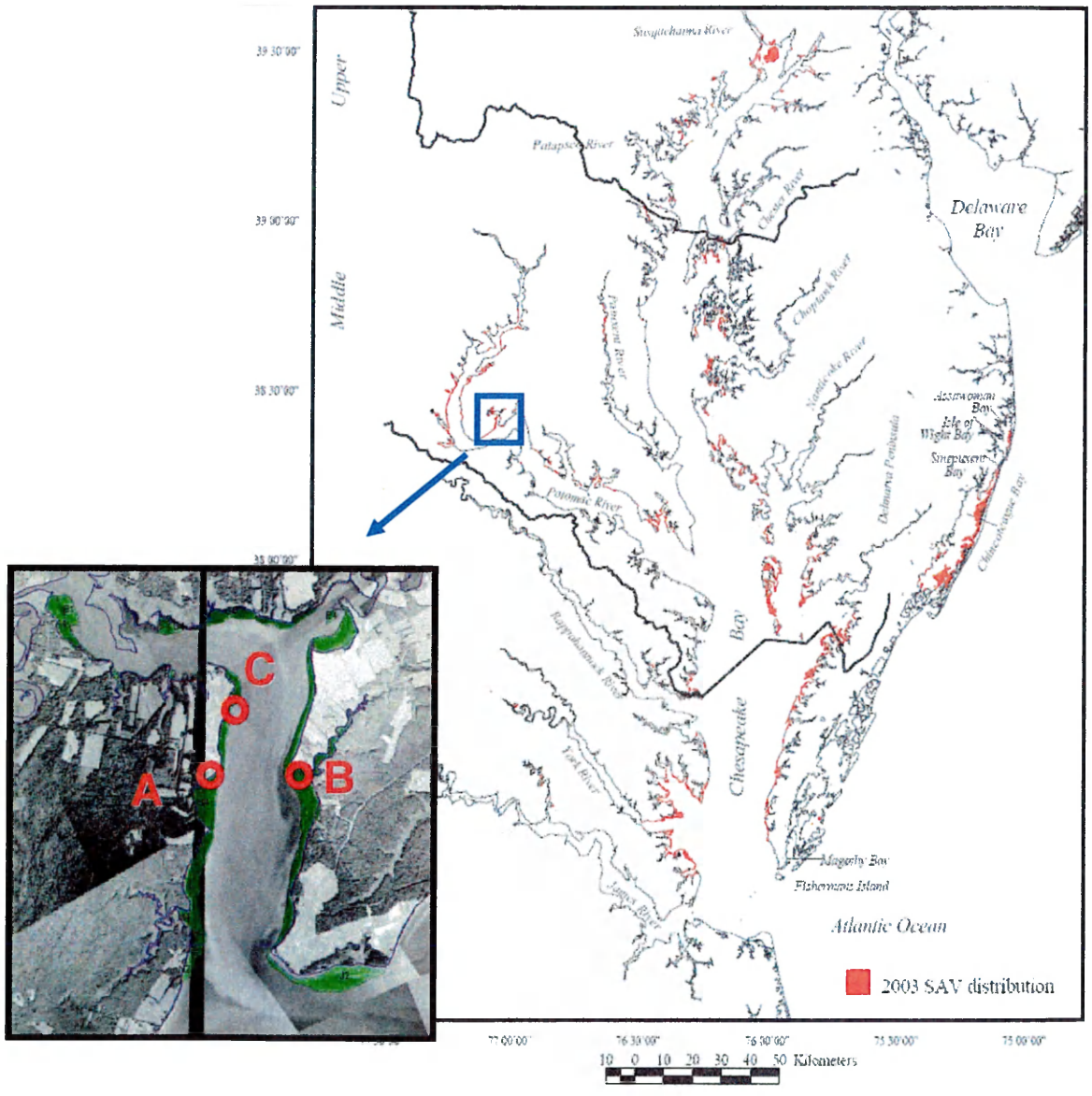

Susquehanna River
Upper
Middle
Patapsco River
Choptank River
Nanticoke River
Pocomoke River
Delaware Bay
Delmarva Peninsula
Assawoman Bay
Isle of Wight Bay
Sinepuxent Bay
Chincoteague Bay
Potomac River
Rappahannock River
York River
James River
Chesapeake Bay
Magothy Bay
Fisherman's Island
Atlantic Ocean
2003 SAV distribution
A
B
C
39 30'00"
39 00'00"
38 30'00"
77 00'00"
76 30'00"
75 30'00"
75 00'00"
10 0 10 20 30 40 50 Kilometers

Figure 4. Layout for dissolved oxygen and light experiment. Small squares represent individual serum bottles. Letters represent treatments; A = oxygenated, light; B = oxygenated, dark; C = hypoxic, light; D = hypoxic, dark.

A	B	C	B
C	D	A	C
A	A	B	C
C	B	D	A
D	B	D	D

Figure 5. Layout for temperature experiment. Rectangles represent individual aquaria. Numbers in diagram represent temperature (°C) treatment.

13°C	22°C
22°C	25°C
13°C	25°C
25°C	29°C
22°C	29°C
25°C	29°C
13°C	29°C
13°C	22°C

Figure 6. Layout for salinity experiment. Each large rectangle represents one 1.22 m x 2.44 m tank and each small square equals one aquaria. Numbers on the diagram represent the salinity treatments; 1= 0 psu; 2 = 5 psu; 3 = 10 psu; 4 = 15 psu.

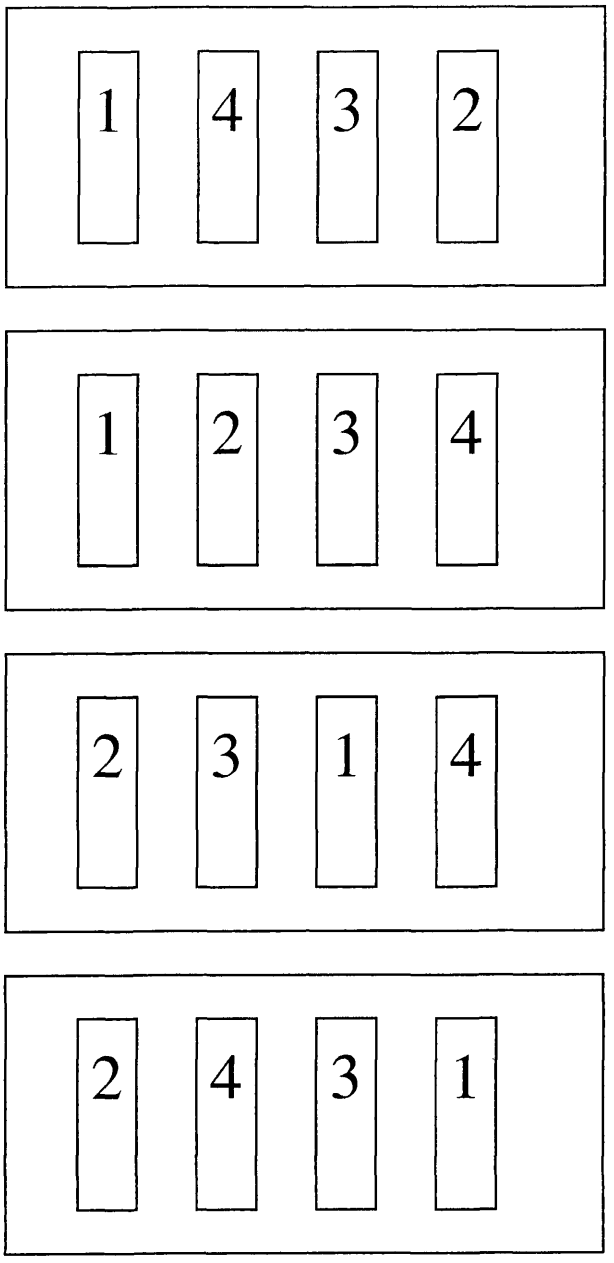

1 4 3 2
1 2 3 4
2 3 1 4
2 4 3 1

Figure 7. Layout for sediment type burial depth experiment. (7A) large rectangles represent 1.22 m x 2.44 m tanks and small rectangles represent individual PVC cores. (7B) Description of treatments assigned to each core (i.e., a core assigned the number 14 has a seed burial depth of 15mm with 25 percent Near Shore (NS) sediment).

TANK

5	12	25	27	10	7
1	14	24	26	19	13
17	4	3	9	6	30
15	2	20	18	23	11
28	8	22	29	21	16

TANK 2

27	14	24	9	20	16
29	25	17	12	4	10
15	26	19	18	6	3
2	8	7	1	13	21
22	5	30	23	11	28

TANK 1

7	10	11	6	1	29
14	22	17	25	18	28
13	20	16	4	12	3
30	27	8	24	9	21
26	2	5	23	15	19

A

Seed Burial Depth (mm)

% NS	2	7	15	25	50	10
10	1	6	11	16	21	26
75	2	7	12	17	22	27
50	3	8	13	18	23	28
25	4	9	14	19	24	29
0	5	10	15	20	25	30

B

Figure 8. Water temperature (°C) for all sampling sites in Nanjemoy Creek from April to October 2004.

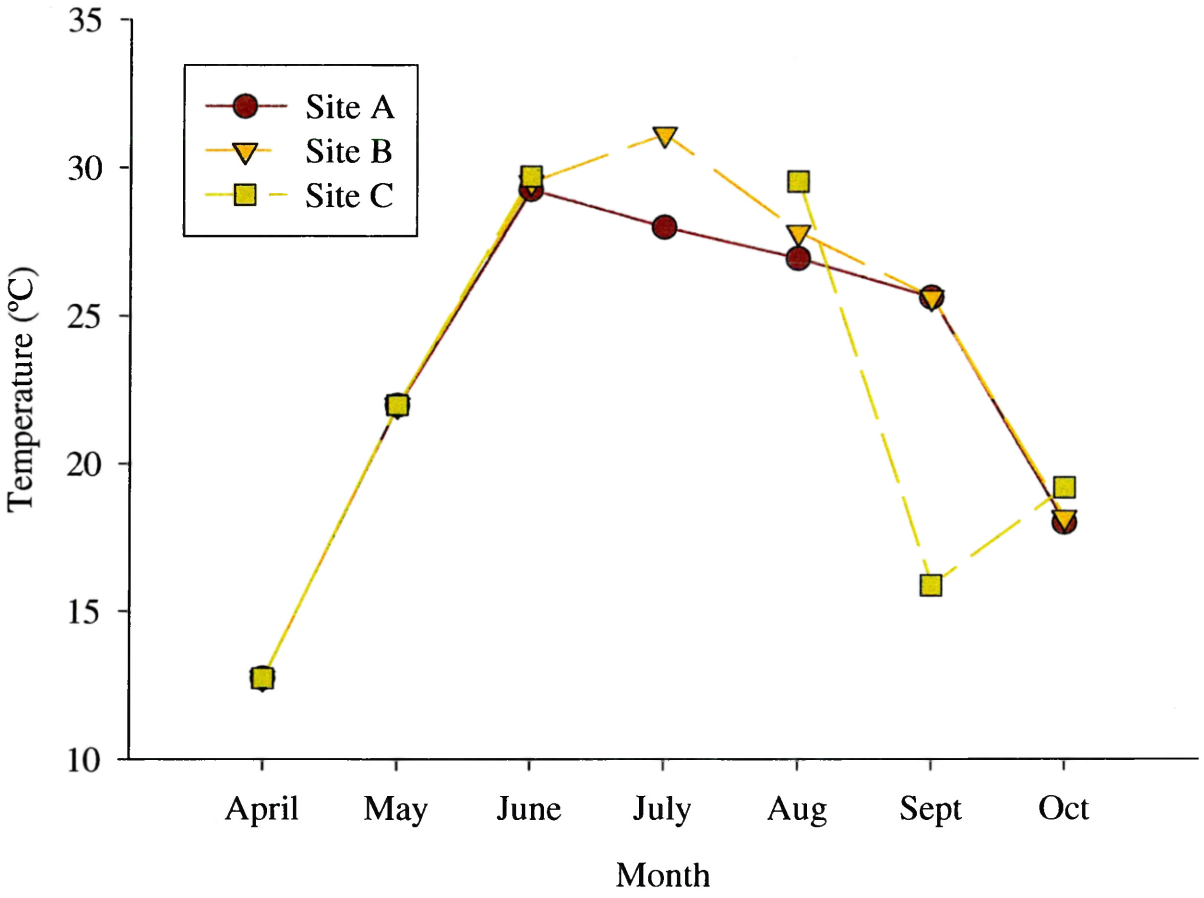

35
30
25
20
15
10
Temperature (°C)
Site A
Site B
Site C
April
May
June
July
Aug
Sept
Oct
Month

Figure 9. Salinity (psu) for all sampling sites in Nanjemoy Creek from April to October 2004.

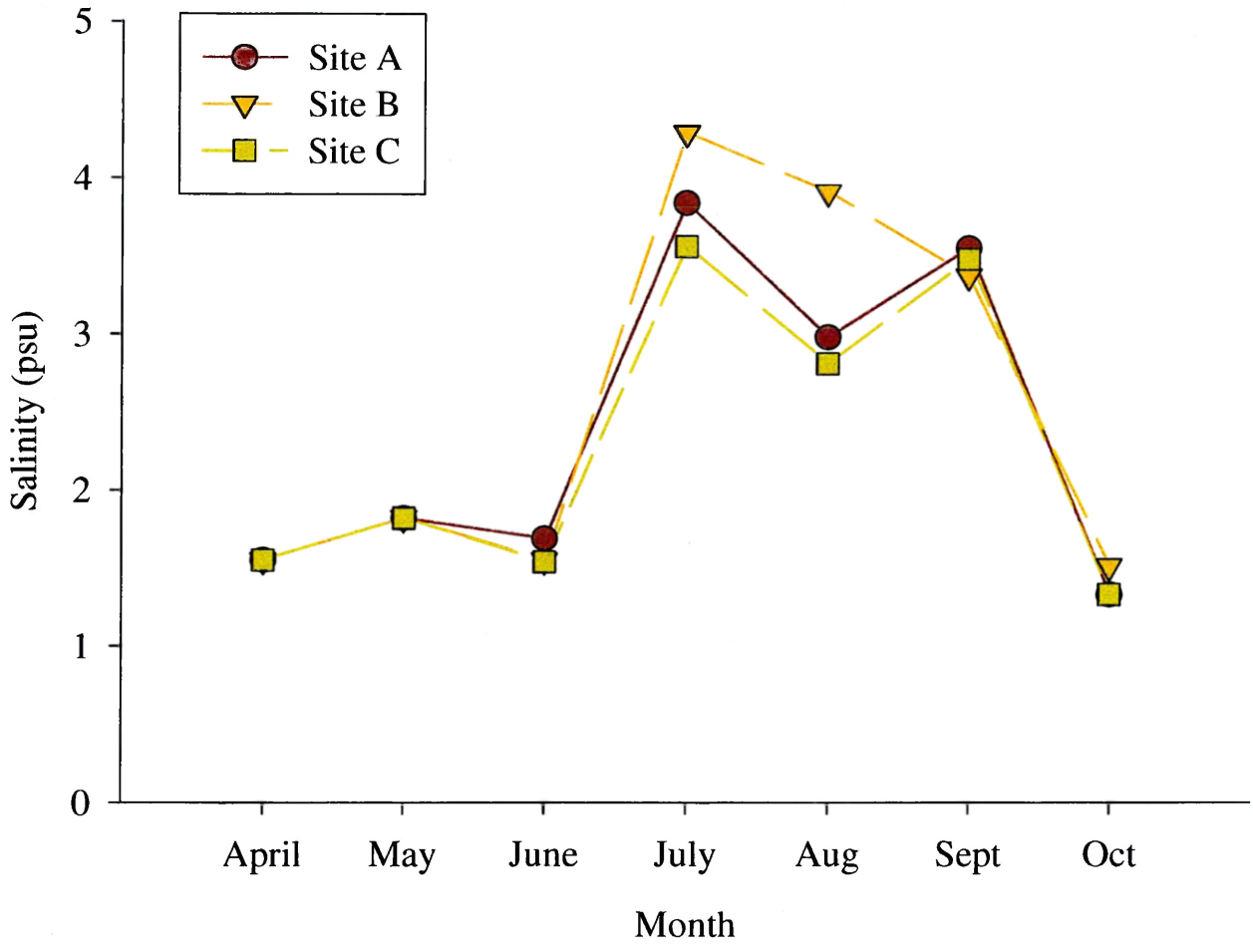

Site A
Site B
Site C
Salinity (psu)
0
1
2
3
4
5
April
May
June
July
Aug
Sept
Oct
Month

Figure 10. Redox (Eh) profiles of sediment cores collected from all Nanjemoy Creek sampling sites in April 2004.

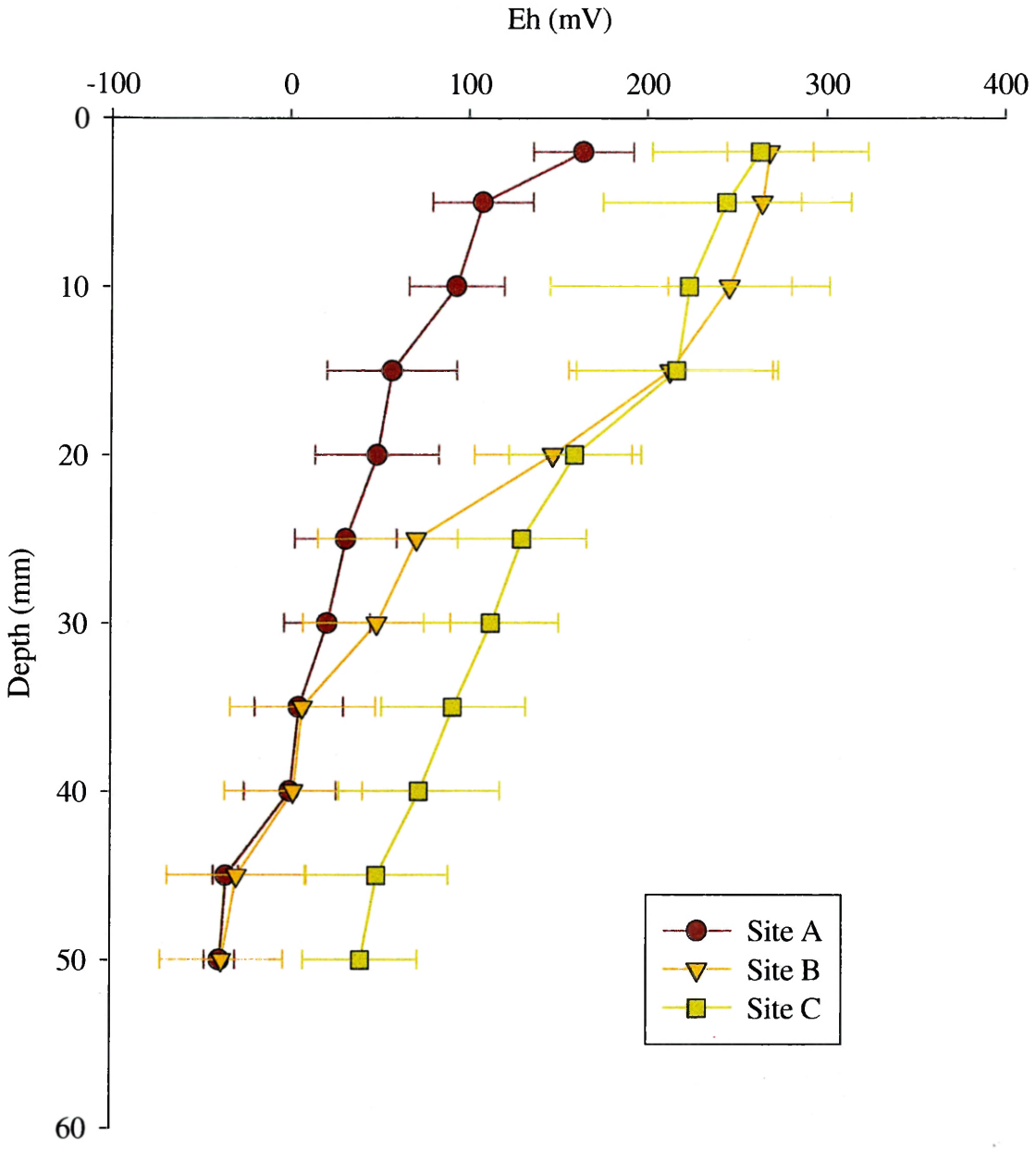

Eh (mV)
-100
0
100
200
300
400
0
10
20
30
40
50
60
Depth (mm)
Site A
Site B
Site C

Figure 11. Total biomass of shoots collected in Nanjemoy Creek between April and October 2004.

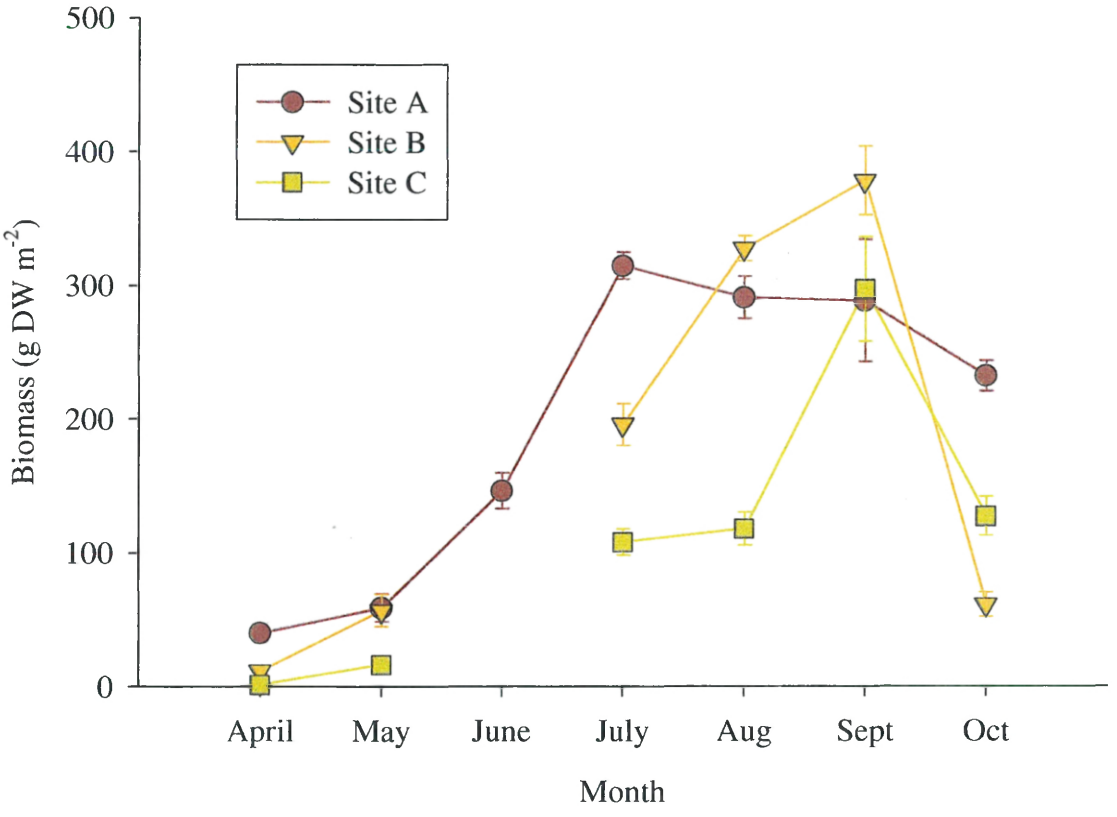

500
400
300
200
100
0
Biomass (g DW m-2)
Site A
Site B
Site C
April
May
June
July
Aug
Sept
Oct
Month

Figure 12. Above and below ground biomass production of *V. americana* over 2004 growing season in Nanjemoy Creek.

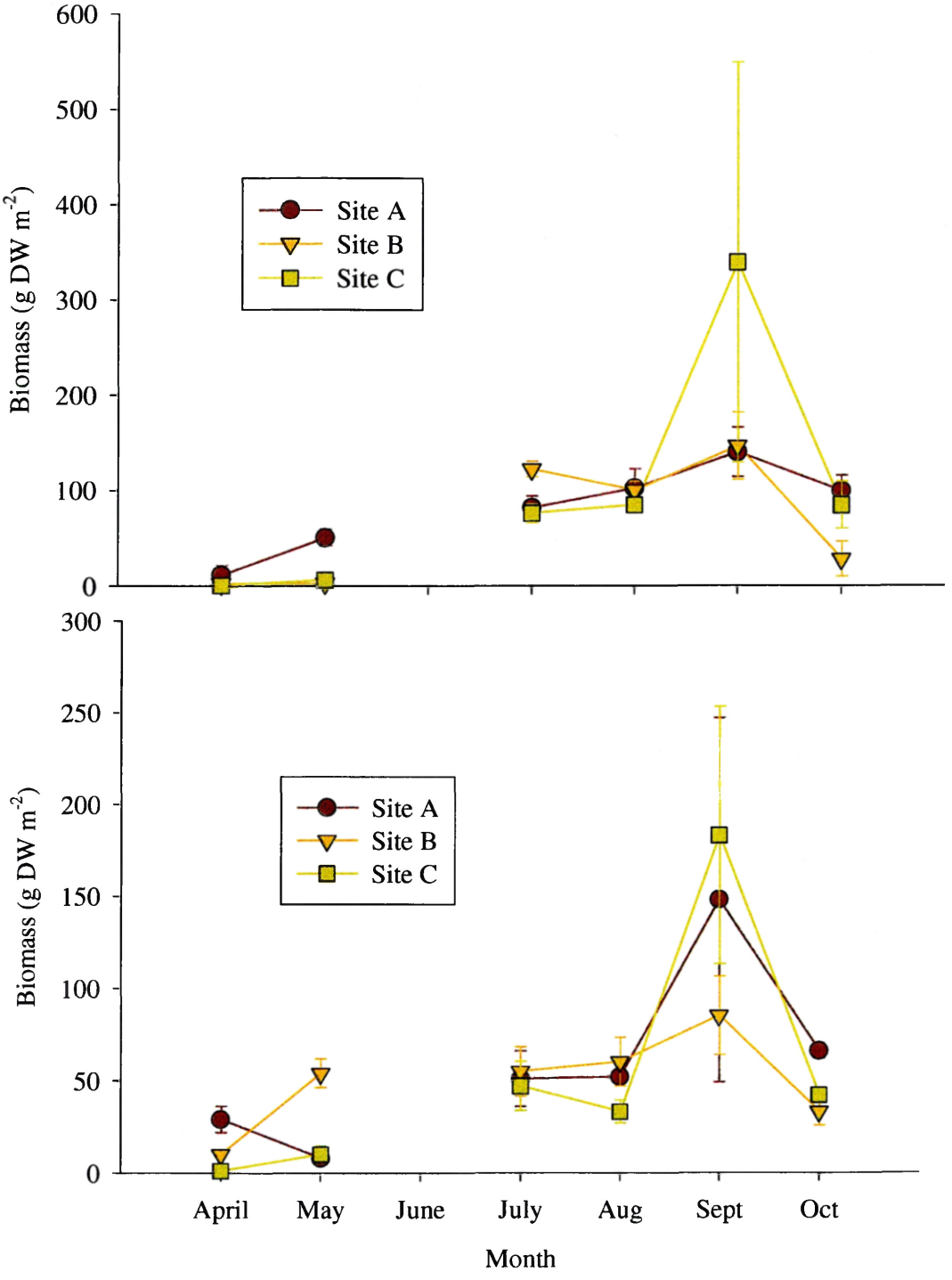

600
500
400
300
200
100
0
Biomass (g DW m-2)
Site A
Site B
Site C
300
250
200
150
100
50
0
Biomass (g DW m-2)
Site A
Site B
Site C
April
May
June
July
Aug
Sept
Oct
Month

Figure 13. Redox (Eh) profiles of sediment cores representing all sediment types in sediment type burial depth experiment.

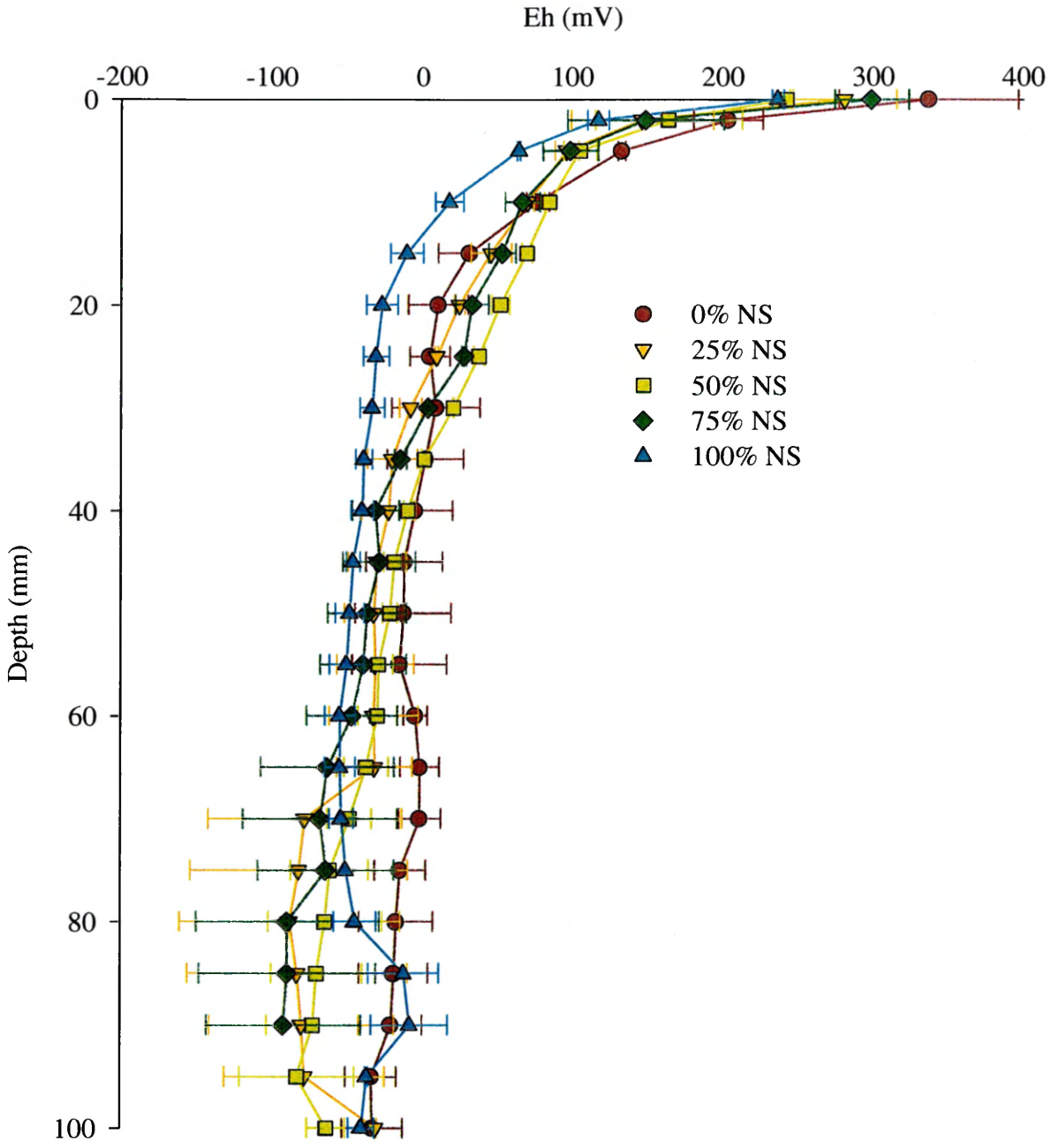

Eh (mV)
-200
-100
0
100
200
300
400
0
20
40
60
80
100
Depth (mm)
0% NS
25% NS
50% NS
75% NS
100% NS

Figure 14. Cumulative germination curve for seeds in dissolved oxygen and light experiment. Final percent germination for oxygenated treatments was 78 percent and 45 percent for hypoxic treatments.

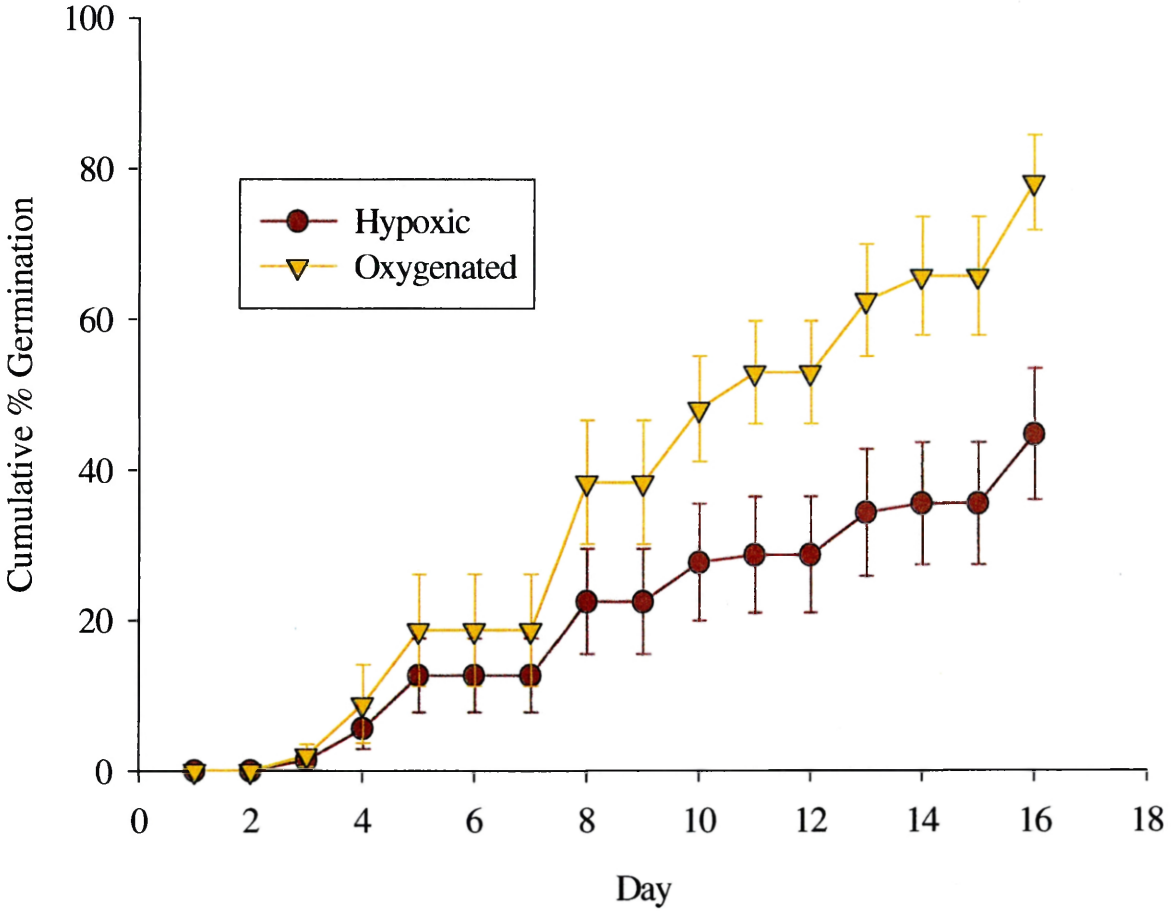

Cumulative % Germination
100
80
60
40
20
0
Hypoxic
Oxygenated
0
2
4
6
8
10
12
14
16
18
Day

Figure 15. Cumulative germination curve for seeds in salinity experiment. 0 and 5 psu curves are significantly different than curves for 10 and 15 psu. Final percent germination for 0 psu = 75 percent, 5 psu = 63 percent; 10 psu = 18 percent and 15 psu = 9 percent.

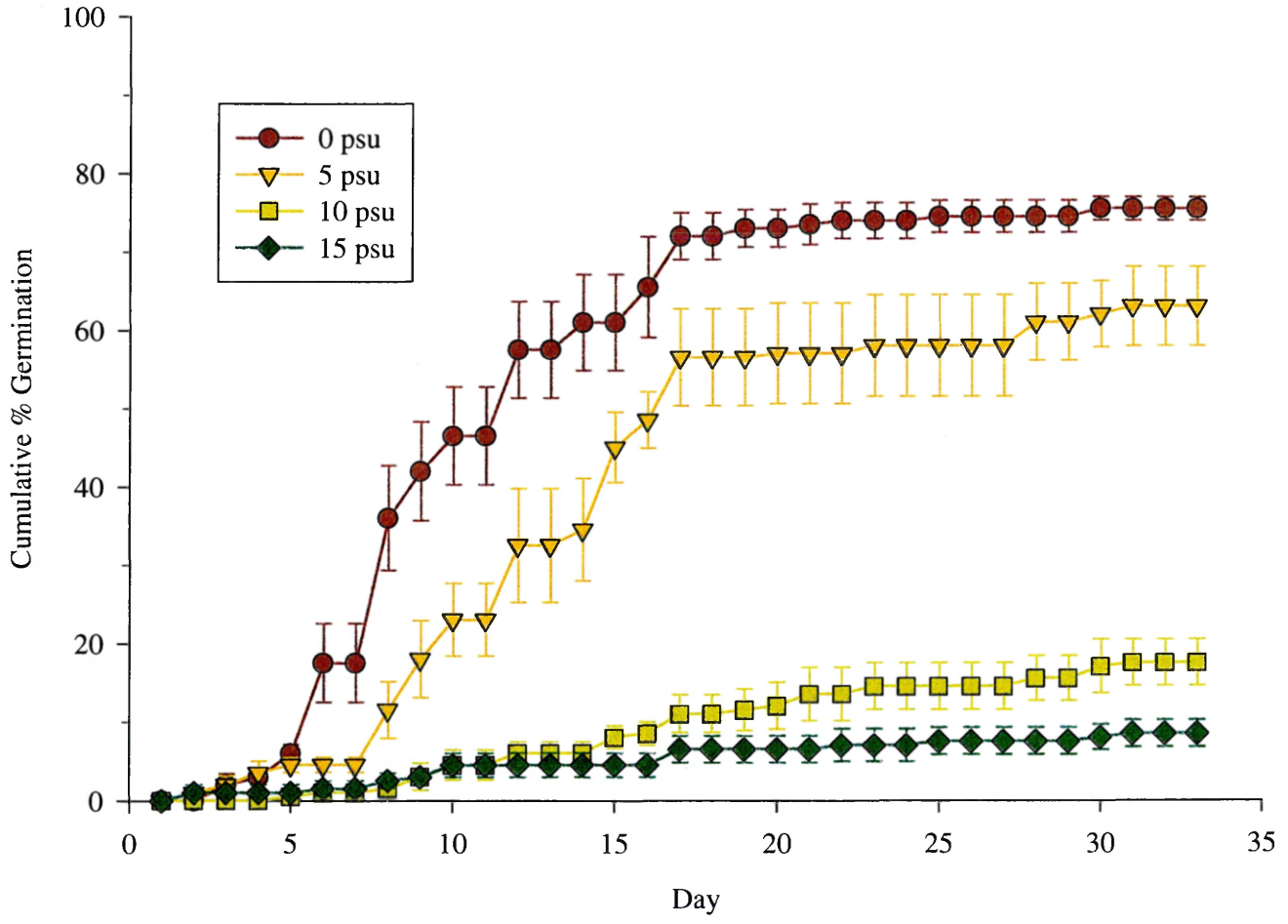

100
80
60
40
20
0
Cumulative % Germination
0 psu
5 psu
10 psu
15 psu
0
5
10
15
20
25
30
35
Day

Figure 16. Final percent germination seeds in salinity experiment. Regression analysis indicates a linear decrease in germination with an increase in salinity ($r^2 = 0.93$).

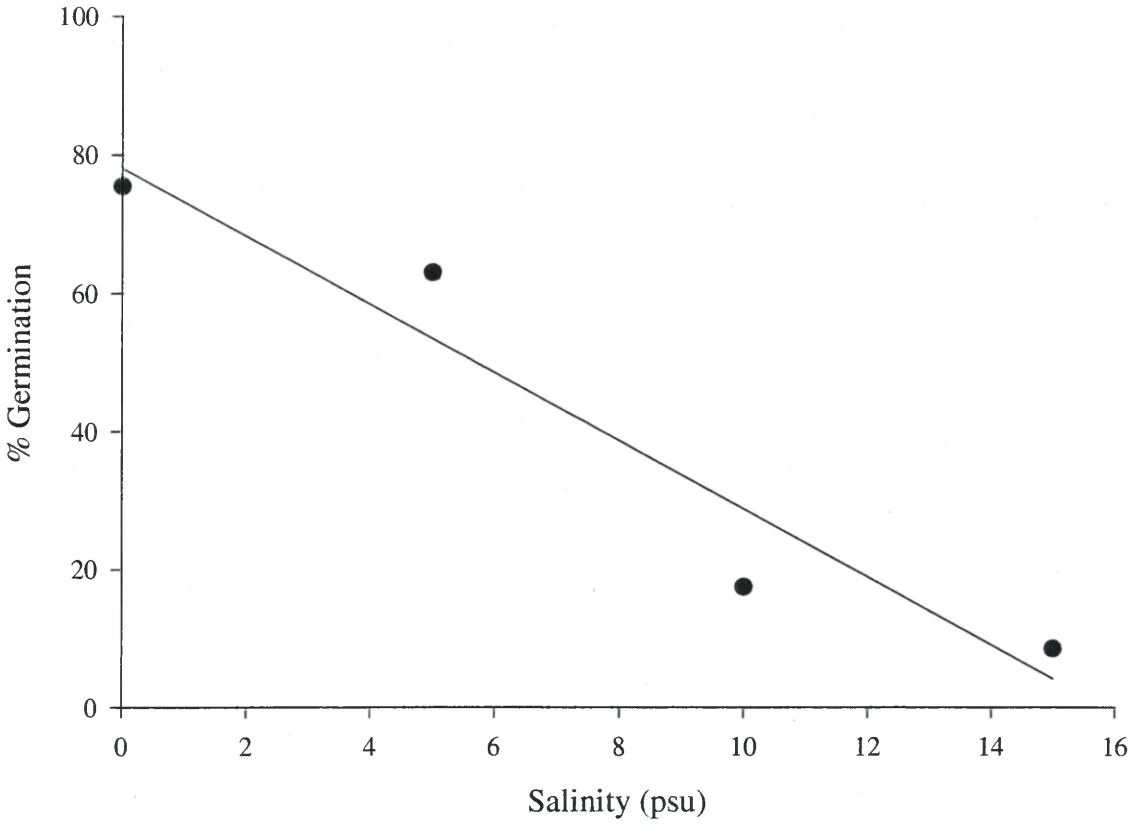

100
80
60
40
20
0
% Germination
0
2
4
6
8
10
12
14
16
Salinity (psu)

Figure 17. Mean time to germination (MTG) for seeds in salinity experiment. MTG for 0 psu = 15 d, 5 psu = 20 d, 10 psu = 29 d, and 15 psu = 30 d. Regression analysis indicates a linear increase in time to germination with an increase in salinity ($r^2 = 0.91$).

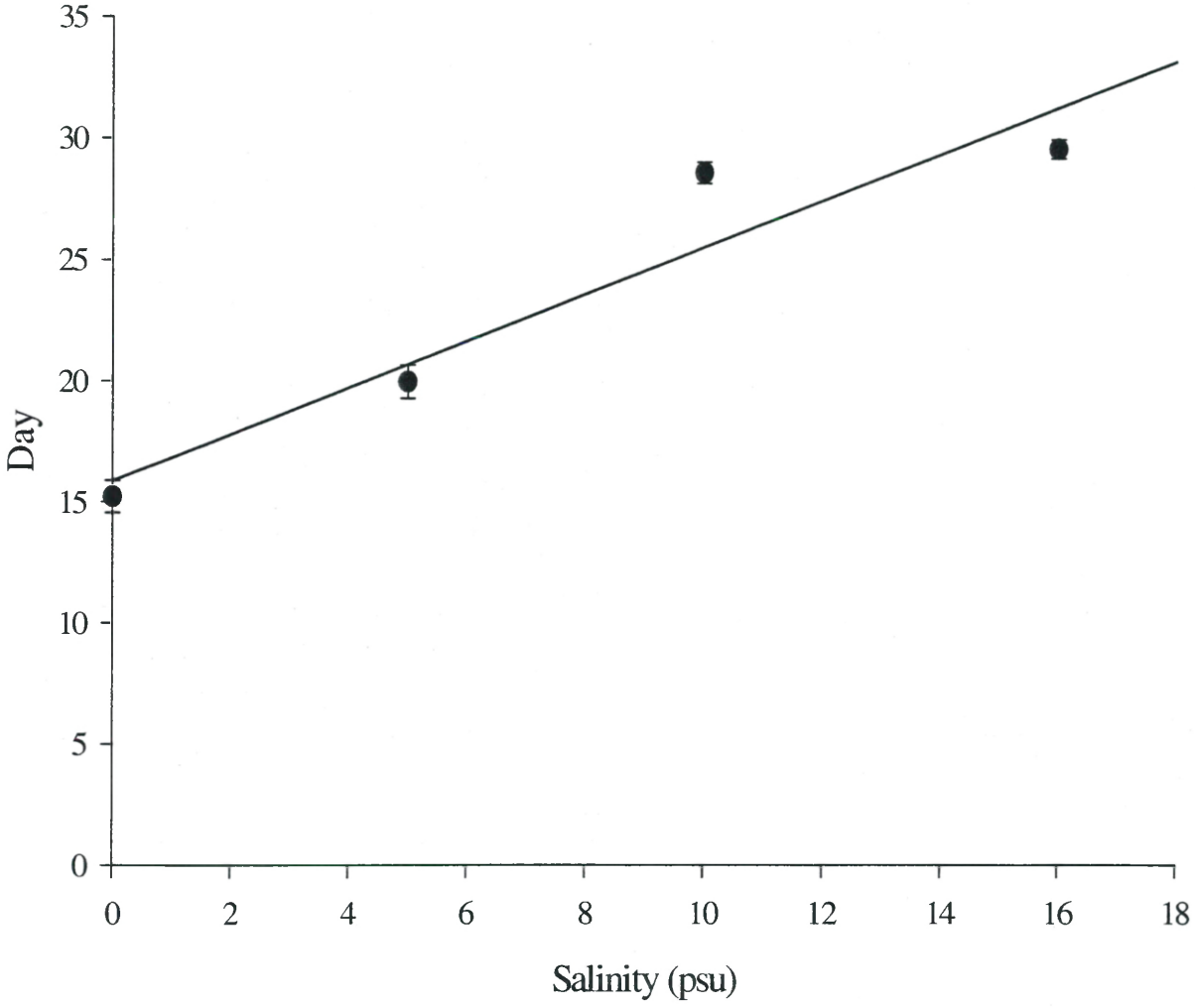

35
30
25
20
15
10
5
0
Day
0
2
4
6
8
10
12
14
16
18
Salinity (psu)

Figure 18. Cumulative germination curve for seeds in temperature experiment. Final percent germination for 13 °C = 3 percent, 22 °C = 73 percent, 25 °C = 86.5 percent, and 29 °C = 97 percent.

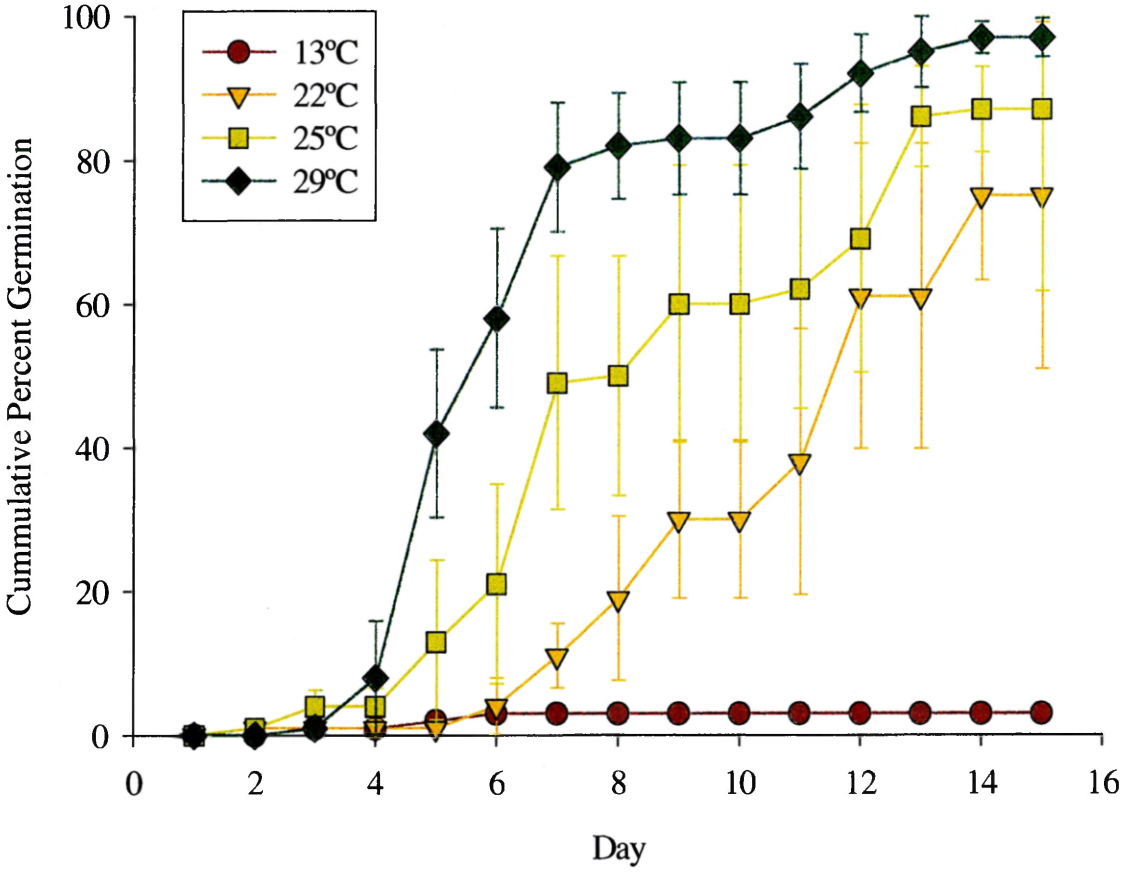

13°C
22°C
25°C
29°C
Cummulative Percent Germination
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
Day

Figure 19. Final percent germination seeds in temperature experiment. Regression analysis indicates a linear increase in germination with temperature ($r^2 = 0.95$).

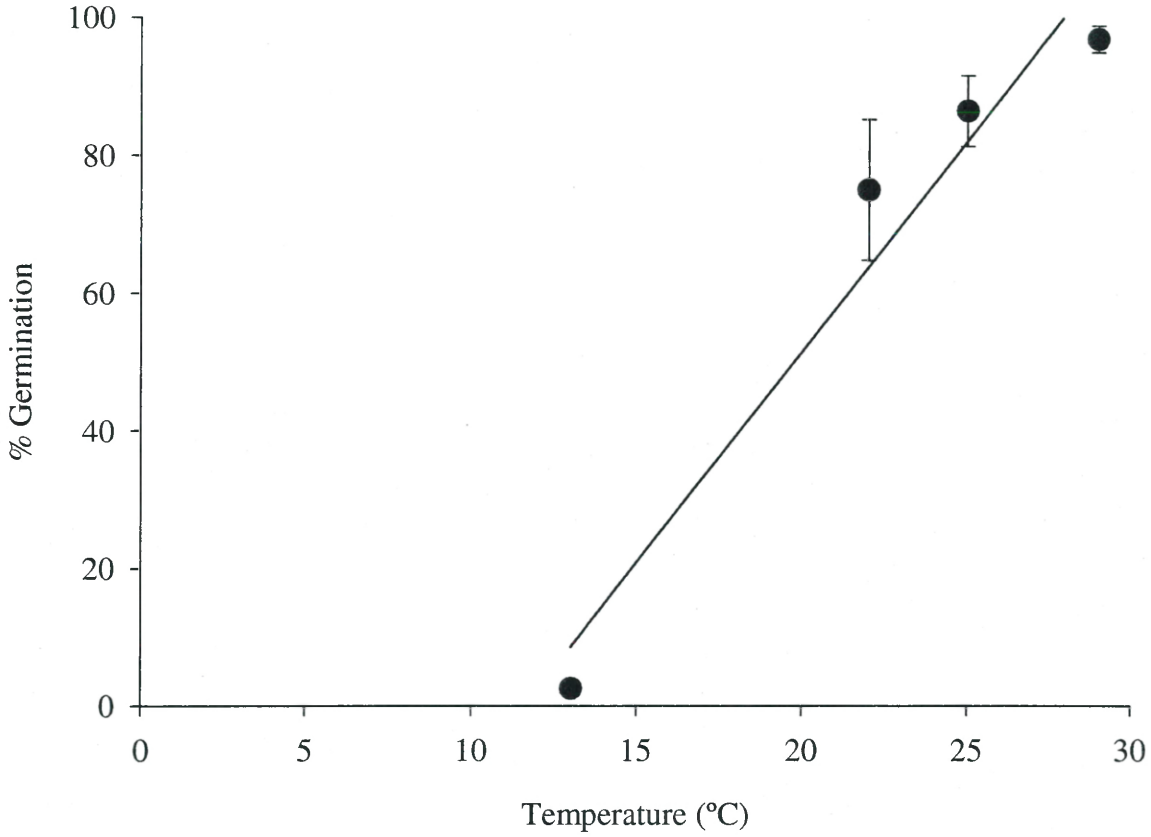

100
80
60
40
20
0
% Germination
0
5
10
15
20
25
30
Temperature (°C)

Figure 20. Mean time to germination (MTG) for seeds in temperature experiment. MTG was not calculated for 13 °C treatments due to lack of germination. MTG for 22 °C = 12 d, 25 °C = 9 d, and 29 °C = 6 d. Regression analysis indicates a linear decrease in time to germination and temperature ($r^2 = 0.99$).

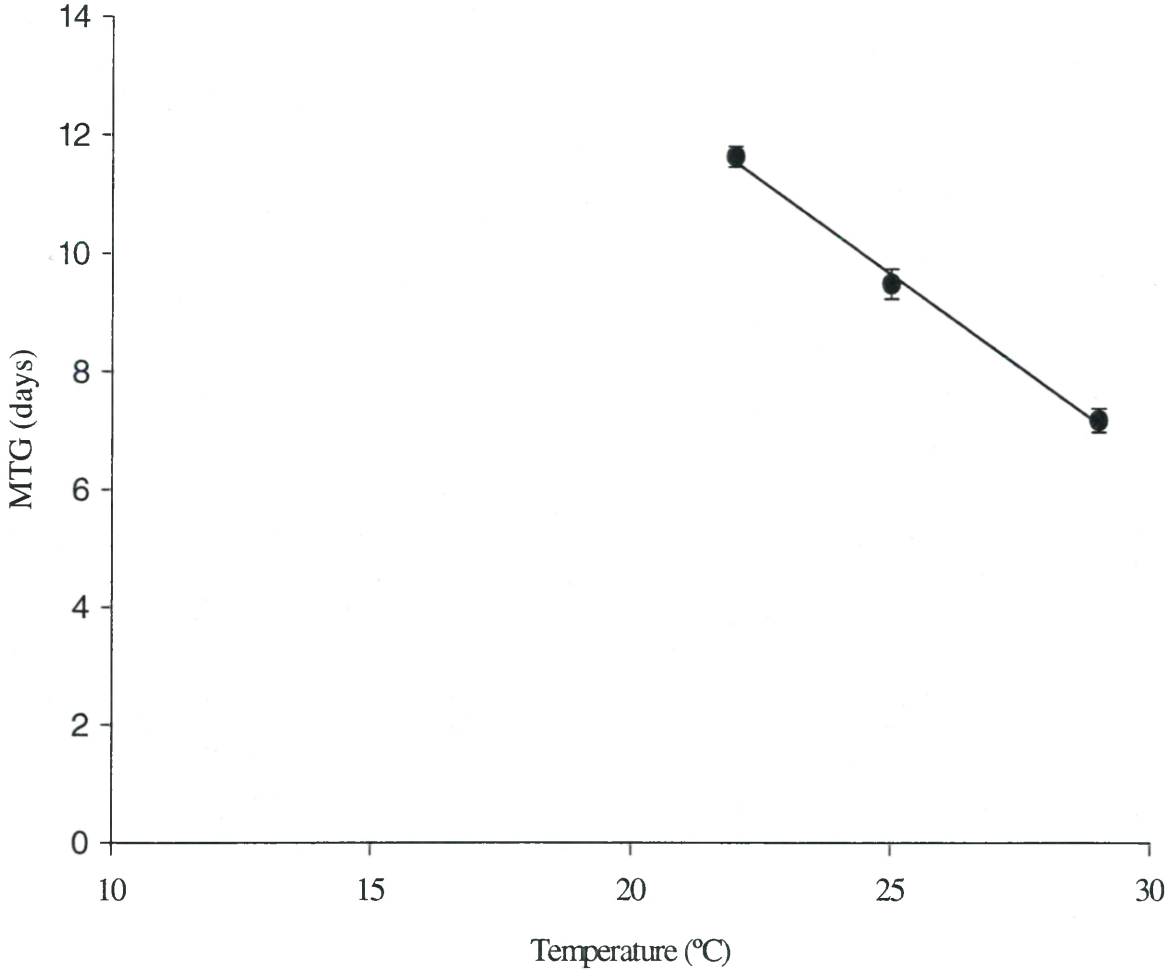

14
12
10
8
6
4
2
0
MTG (days)
10
15
20
25
30
Temperature (°C)

Figure 21. Cumulative germination curves for seeds in the sediment type burial depth experiment. Final percent germination for 100 percent = 17 percent, 75 percent = 13 percent, 50 percent = 8 percent, 25 percent = 3 percent, and 0 percent = <1 percent.

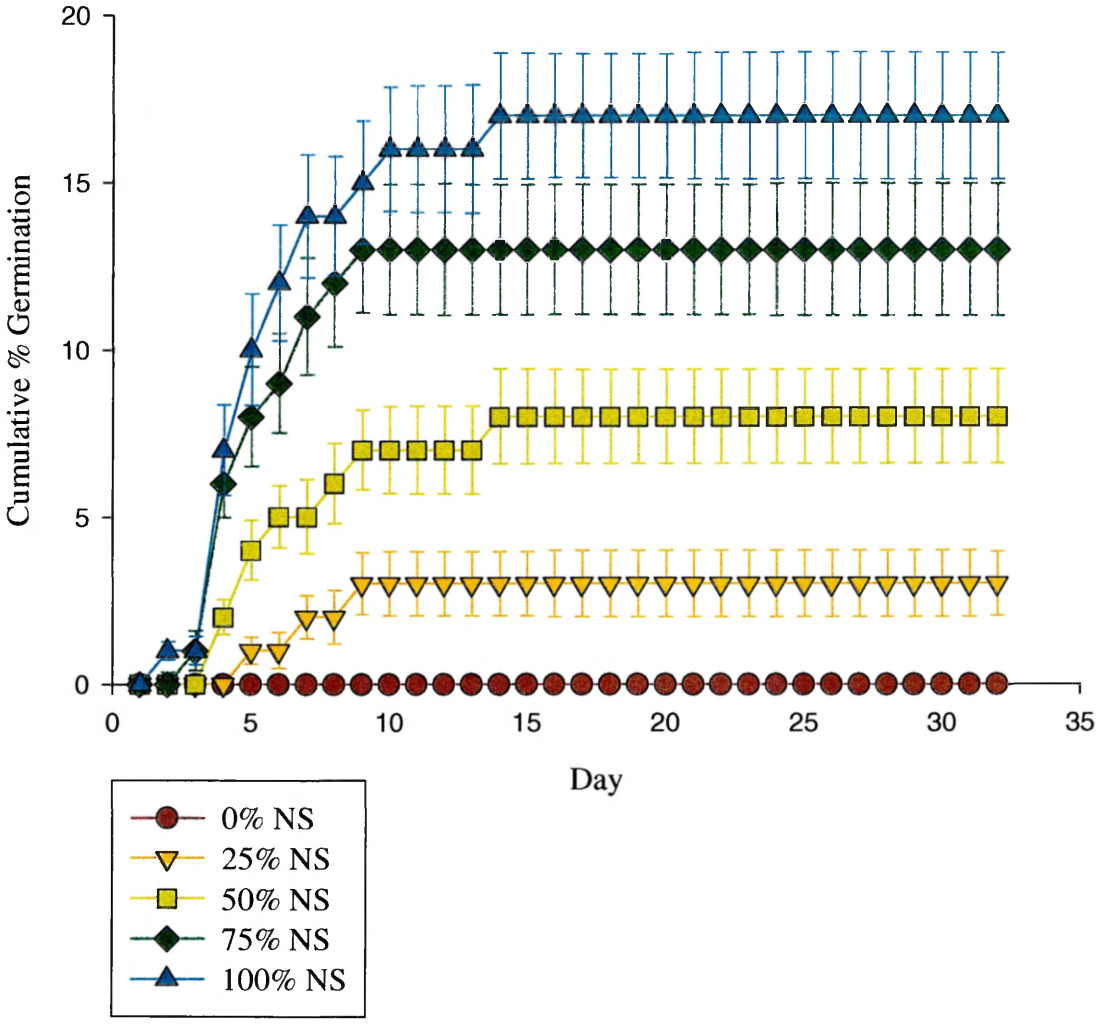

Cumulative % Germination
20
15
10
5
0
0
5
10
15
20
25
30
35
Day
0% NS
25% NS
50% NS
75% NS
100% NS

Figure 22. Final percent germination seeds in sediment type burial depth experiment. Regression analysis indicates a linear increase in germination with an increase in percent NS sediment ($r^2 = 0.99$).

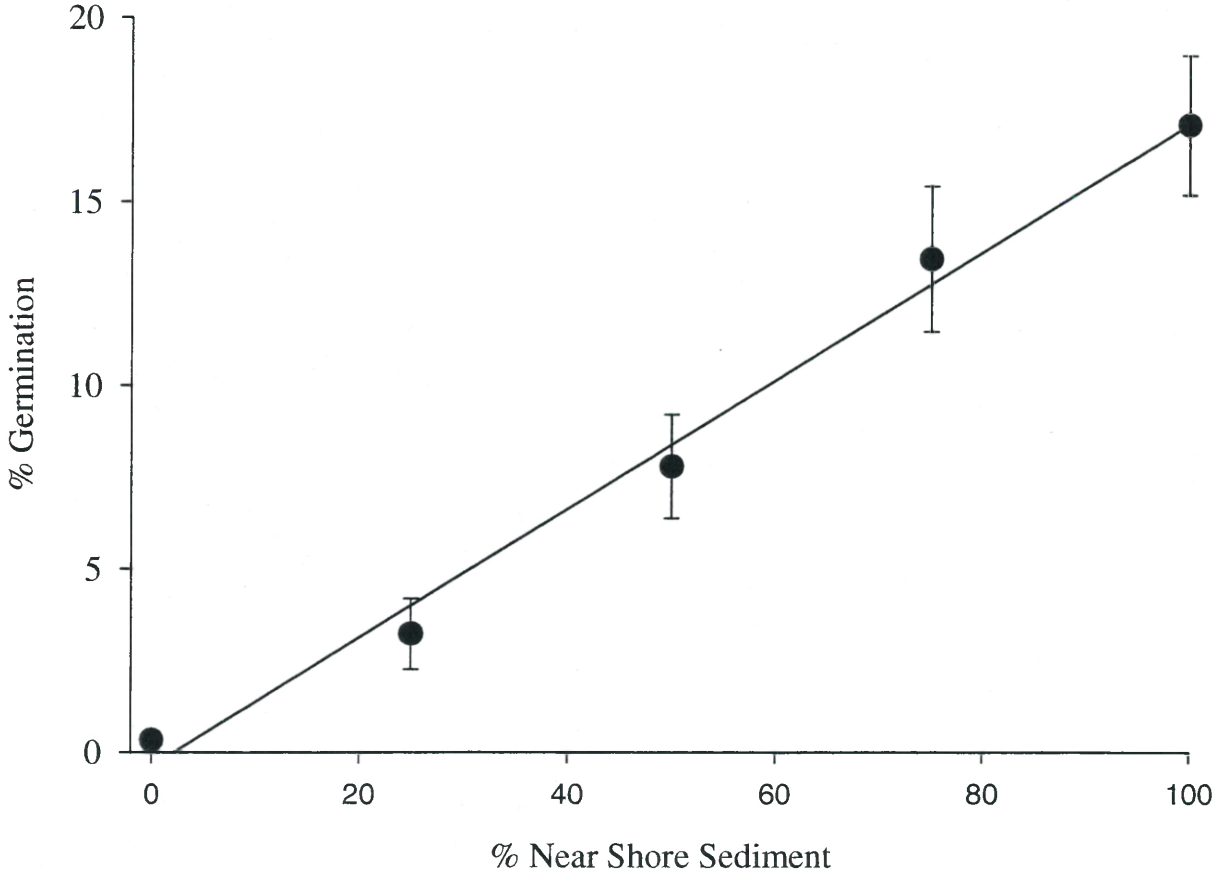

20
15
10
5
0
% Germination
0
20
40
60
80
100
% Near Shore Sediment

Figure 23. Final percent germination seeds in sediment type burial depth experiment based on sediment sand content. Regression analysis indicates an exponential increase in germination with increasing sand content ($r^2 = 0.99$).

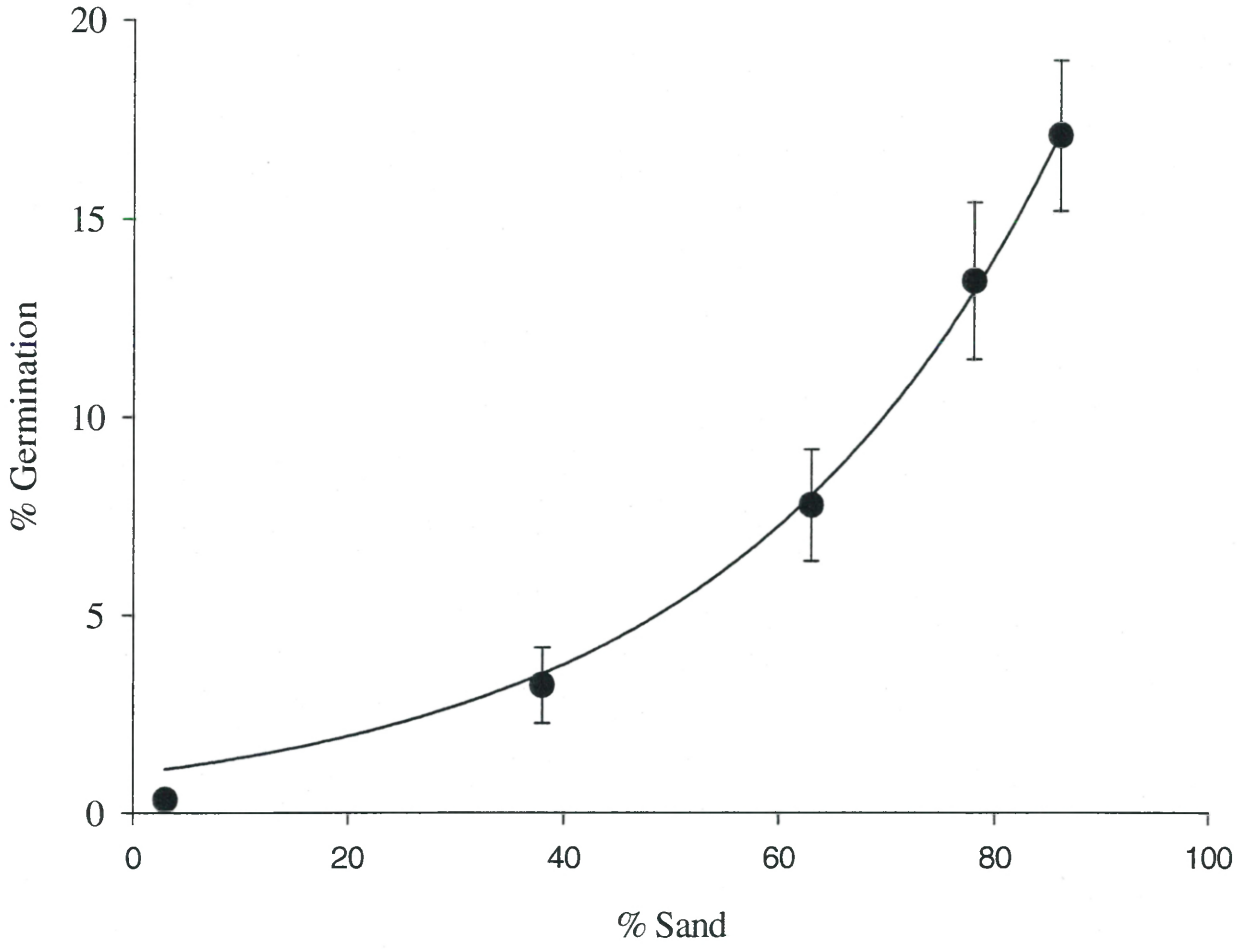

20
15
10
5
0
% Germination
0
20
40
60
80
100
% Sand

Figure 24. Final percent germination of seeds in sediment type burial depth experiment based on sediment organic content. Regression analysis indicates an exponential decrease in germination with increasing organic content ($r^2 = 0.96$).

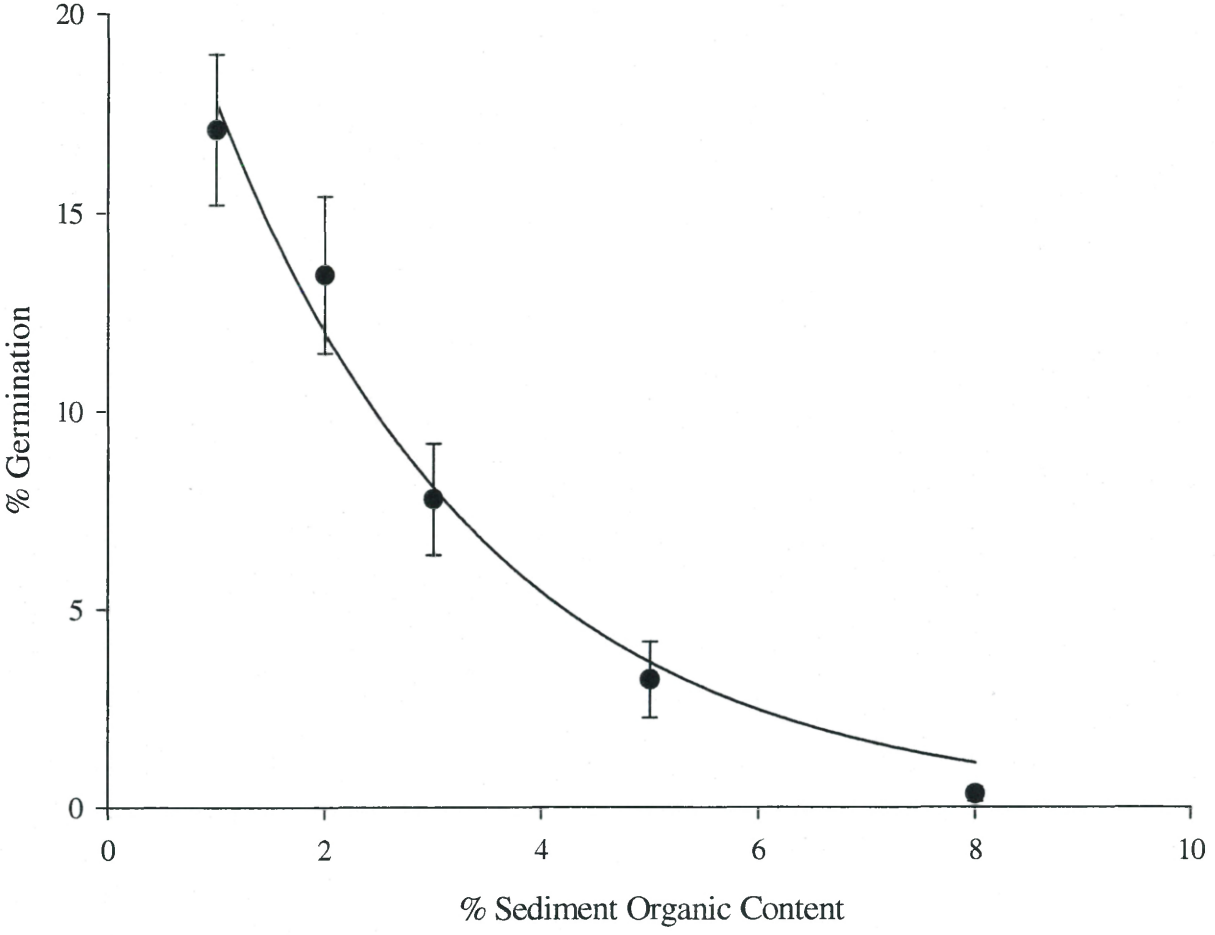

20
15
10
5
0
% Germination
0
2
4
6
8
10
% Sediment Organic Content

Figure 25. Mean time to germination (MTG) for seeds in sediment type burial depth experiment. MTG for 100 percent NS = 28 d, 75 percent NS = 29 d, 50 percent NS = 30 d, 25 percent NS = 31 d, 0 percent NS = 32 d. Regression analysis indicates a linear decrease in time to germination with an increase in percent NS sediment ($r^2 = 0.98$).

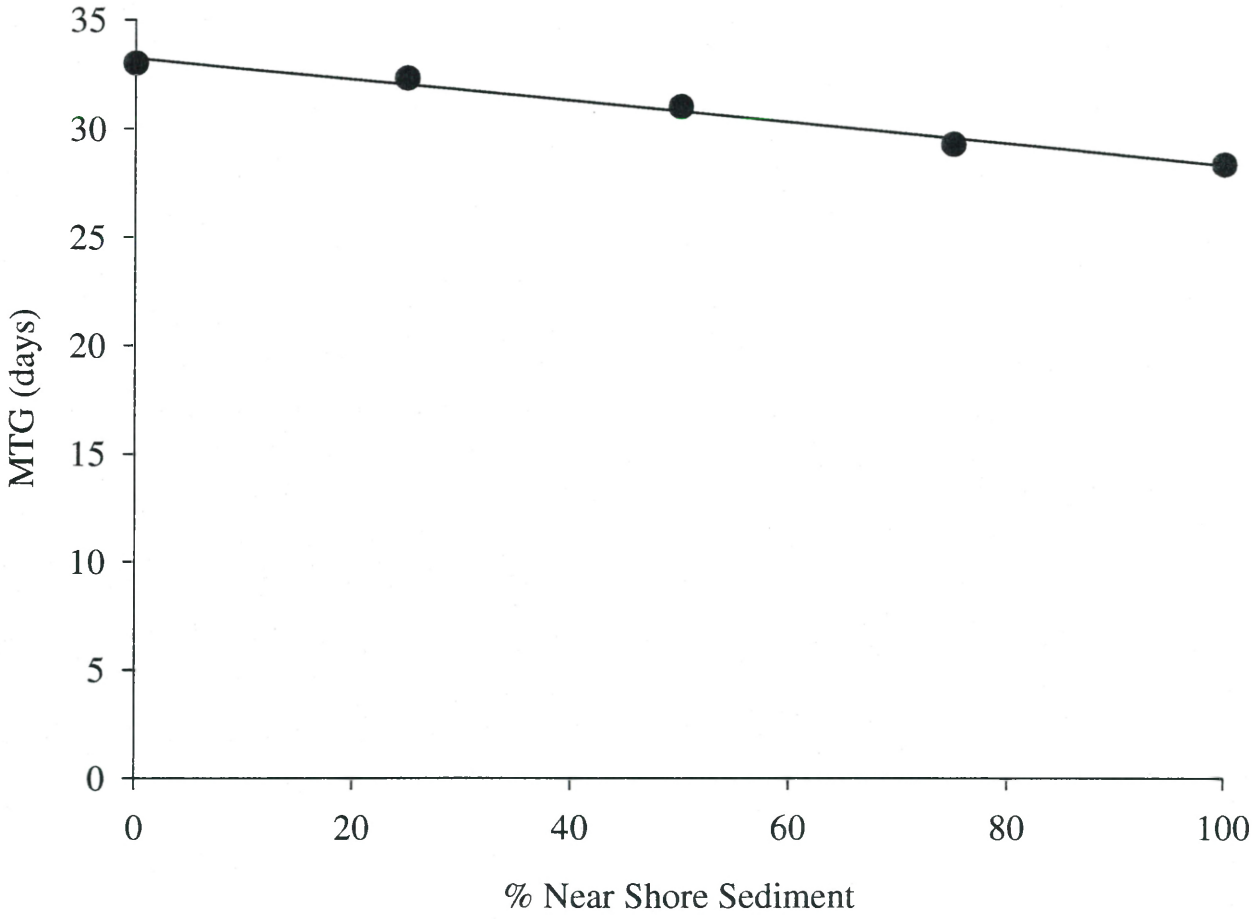

35
30
25
20
15
10
5
0
MTG (days)
0
20
40
60
80
100
% Near Shore Sediment

Figure 26. Mean time to germination (MTG) for seeds in sediment type burial depth experiment based on percent sand in sediment. Regression analysis indicates a linear decrease in time to germination with an increase in sediment sand content ($r^2 = 0.94$).

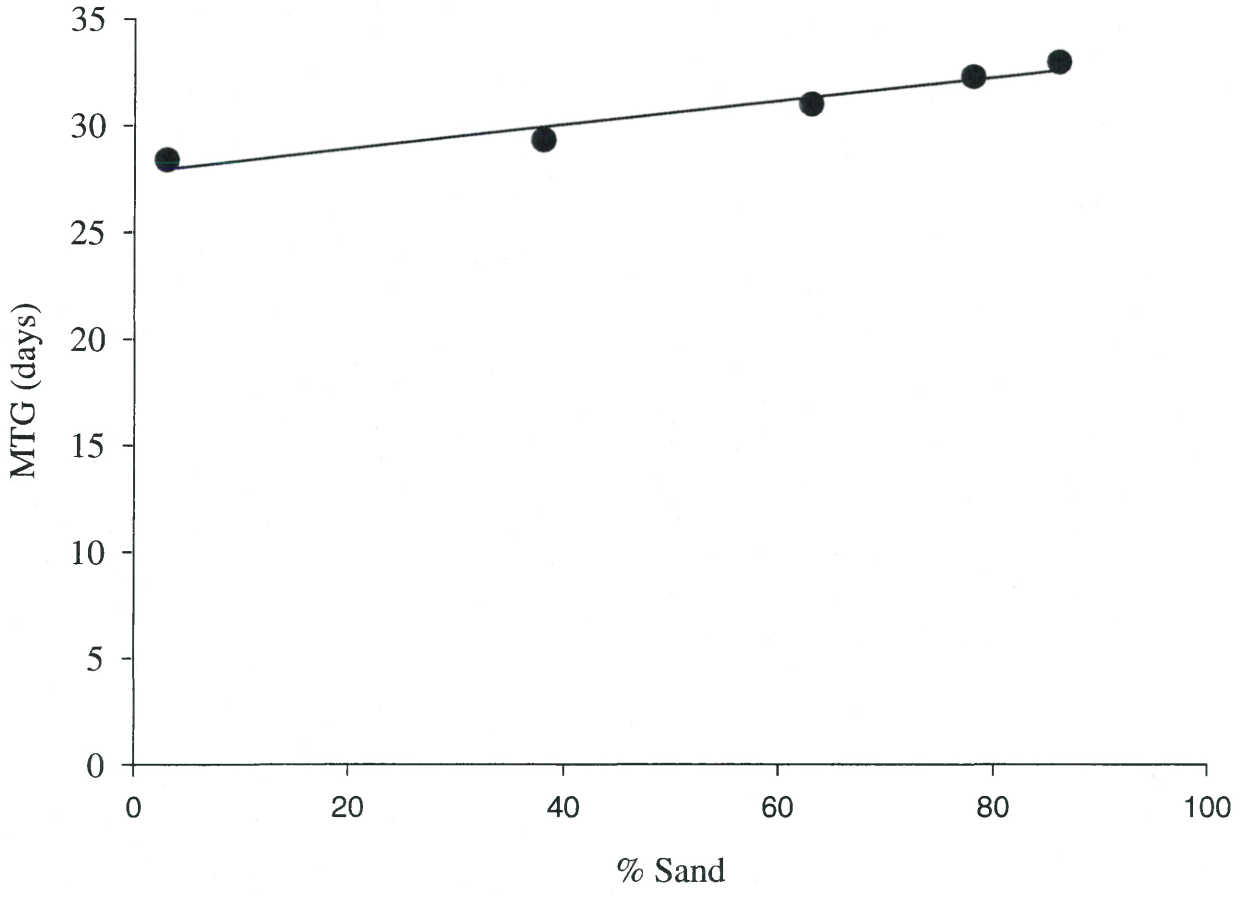
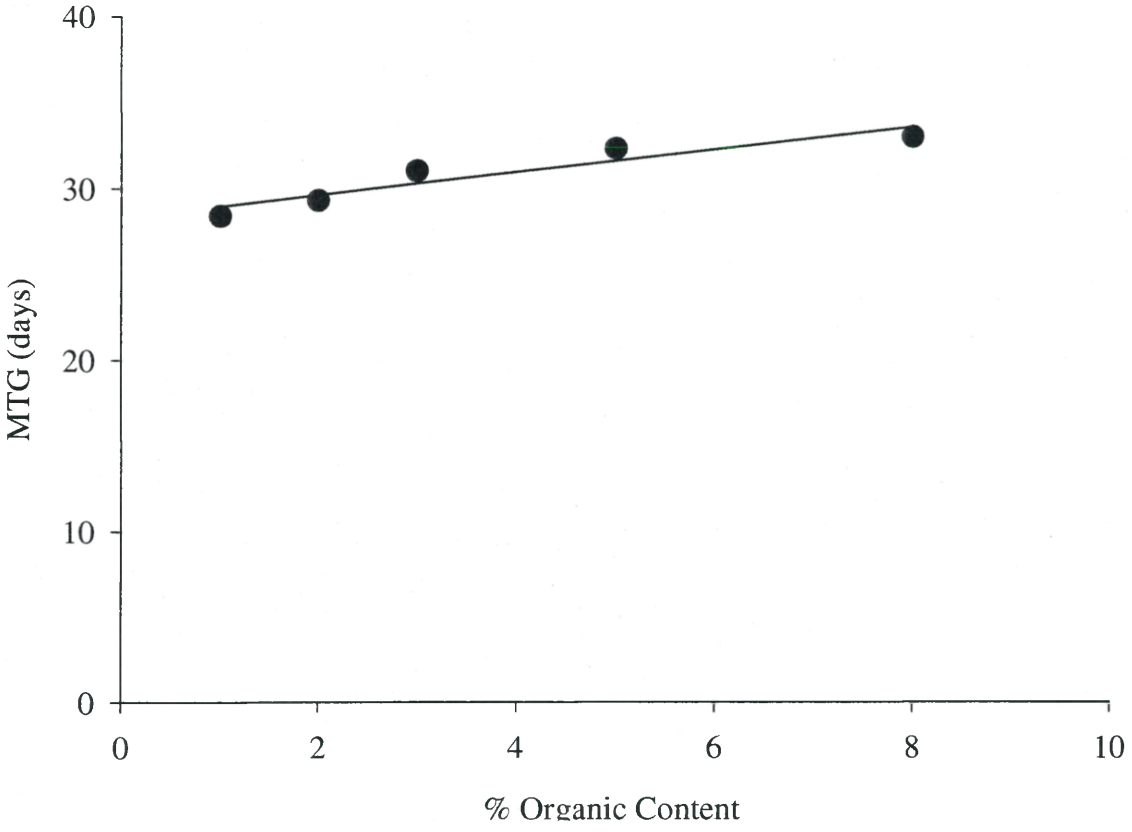

35
30
25
20
15
10
5
0
MTG (days)
0
20
40
60
80
100
% Sand

Figure 27. Mean time to germination (MTG) for seeds in sediment type burial depth experiment based on percent organic content in sediment. Regression analysis indicates a linear increase in time to germination with an increase of sediment organic content (r^2 = 0.88).


40
30
20
10
0
MTG (days)
0
2
4
6
8
10
% Organic Content

APPENDIX 1

Water quality data from Nanjemoy Creek throughout the entire 2004 *V. americana* growing season (April-October). * Data are average values from Chesapeake Bay Program water quality monitoring stations RET2.2 and RET2.4 located at the mouth of Nanjemoy Creek in the Potomac River.

Date mm/dd/yy	Site	Water Depth cm	K_d m^{-2}	DO mg L^{-1}	Salinity psu	H_2O Temp °C	Chl a ug L^{-1}	TSS mg L^{-1}
04/12/04	*	100	.	10.61	1.55	12.7	5.16	21.39
05/10/04	*	100	.	8.67	1.82	19.5	12.05	41.51
06/18/04	A	77	2.3	11.44	1.69	29.3	11.42	9.27
06/18/04	B	74	1.5	9.31	1.55	29.5	38.29	3.84
06/18/04	C	56	1.2	6.84	1.54	29.7	23.95	17.31
07/21/04	A	60	2.4	9.5	3.84	28	10.06	19.97
07/21/04	B	60	3.6	11.1	4.29	31.1	9.02	6.81
07/21/04	C	100	2.0	7.83	3.56	26.9	14.04	35.63
08/19/04	A	100	0.8	8.93	2.98	27.8	9.82	19.17
08/19/04	B	70	2.0	11.83	3.91	29.5	43.04	17.56
08/19/04	C	66	1.4	8.33	2.81	25.6	11.7	34.49
09/14/04	A	100	1.7	8.97	3.55	25.6	54.45	8.81
09/14/04	B	85	.	8.71	3.37	18.6	31.04	15.52
09/14/04	C	60	2.4	8.31	3.48	18	34.19	19.96
10/12/04	A	70	.	11.21	1.33	18.2	10.02	38.25
10/12/04	B	70	.	9.75	1.51	19.2	10.22	22.98
10/12/04	C	70	2.2	9.43	1.33	.	9.05	26.35

APPENDIX 2

Average water column nutrient concentrations for all sites in Nanjemoy Creek from June to October. Water column nutrients were not analyzed for April and May. Concentrations are reported as the mean ± SE.

	Site A	**Site B**	**Site C**
$NO_2^- + NO_3^-$ µM			
June	0.61 ± 0.01	0.11 ± 0.00	0.10 ± 0.02
July	0.11 ± 0.01	0.30 ± 0.04	0.27 ± 0.05
August	0.12 ± 0.01	0.11 ± 0.01	0.09 ± 0.02
September	0.09 ± 0.00	3.44 ± 0.04	0.11 ± 0.01
October	16.07 ± 0.19	17.49 ± 0.72	29.10 ± 0.76
NH_4^+µM			
June	0.74 ± 0.13	0.60 ± 0.03	0.56 ± 0.10
July	0.37 ± 0.04	1.38 ± 0.14	1.30 ± 0.30
August	0.72 ± 0.04	0.67 ± 0.06	0.37 ± 0.05
September	0.57 ± 0.02	1.26 ± 0.06	0.47 ± 0.03
October	1.01 ± 0.07	1.77 ± 0.08	1.06 ± 0.11
PO_4^{-3}µM			
June	0.48 ± 0.03	0.34 ± 0.03	0.57 ± 0.03
July	0.40 ± 0.04	0.44 ± 0.02	0.73 ± 0.02
August	0.75 ± 0.02	0.33 ± 0.01	0.84 ± 0.01
September	0.86 ± 0.02	1.23 ± 0.00	1.28 ± 0.02
October	1.48 ± 0.01	1.03 ± 0.01	1.40 ± 0.01

APPENDIX 3

1-way ANOVA for effects of time on water column nutrient concentrations from samples collected from June to October in Nanjemoy Creek. Numbers in bold are significant; n=5 for all dates.

$NO_2^-+NO_3^-$ µM			
Site A	**DF**	**F**	**p**
Date	4	6821.06	**<0.0001**
Residual	10		
Site B			
Date	4	538.79	**<0.0001**
Residual	10		
Site C			
Date	4	1430.40	**<0.0001**
Residual	10		

NH_4^+µM			
Site A	**DF**	**F**	**p**
Date	4	10.42	**0.0014**
Residual	10		
Site B			
Date	4	36.56	**<0.0001**
Residual	10		
Site C			
Date	4	7.02	**0.0059**
Residual	10		

PO_4^{-3}µM			
Site A	**DF**	**F**	**p**
Date	4	256.37	**<0.0001**
Residual	10		
Site B			
Date	4	579.57	**<0.0001**
Residual	10		.
Site C			
Date	4	311.37	**<0.0001**
Residual	10		

APPENDIX 4

Average sediment organic content for all depths at all sites in Nanjemoy Creek from April to October. Sediment cores were not collected in May. Concentrations are reported as the mean ± SE.

	Site A	Site B	Site C
Organic Content			
April			
2	1.3 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
5	1.3 ± 0.0	1.3 ± 0.0	0.7 ± 0.0
10	1.0 ± 0.0	0.9 ± 0.0	0.7 ± 0.0
June			
2	4.3 ± 0.4	2.3 ± 0.7	0.7 ± 0.2
5	3.8 ± 0.2	1.7 ± 0.5	1.2 ± 0.2
10	4.4 ± 1.4	2.8 ± 1.7	1.5 ± 0.7
July			
2	4.3 ± 0.8	2.2 ± 0.2	2.0 ± 0.9
5	4.8 ± 0.1	1.7 ± 0.2	0.8 ± 0.2
10	4.2 ± 0.6	1.9 ± 0.2	2.2 ± 1.7
August			
2	7.3 ± 0.6	2.2 ± 0.3	1.5 ± 0.4
5	7.4 ± 0.5	2.7 ± 0.7	1.3 ± 0.3
10	6.0 ± 1.3	2.5 ± 0.3	2.9 ± 1.5
September			
2	6.0 ± 0.1	1.9 ± 0.2	1.2 ± 0.1
5	5.3 ± 0.2	1.3 ± 0.2	1.0 ± 0.2
10	4.9 ± 0.2	1.4 ± 0.2	2.0 ± 0.5
October			
2	2.7 ± 0.3	1.7 ± 0.2	1.2 ± 0.4
5	2.0 ± 0.5	1.3 ± 0.2	1.4 ± 0.1
10	2.0 ± 0.5	1.0 ± 0.3	1.7 ± 0.1

APPENDIX 5

2-way ANOVA on effects of sediment depth (0-2, 2-5, 5-10 cm) and type (percent sand, silt, clay) on organic content of cores collected from Nanjemoy Creek. Numbers in bold are significant factors, n=3 for both burial depth and sediment type.

Site A	**DF**	**F**	**p**
Depth	2	0.01	0.9895
Type	2	68.71	**0.0001**
Depth*Type	4	8.82	**0.0035**
Residual	9		
Site B	**DF**	**F**	**p**
Depth	2	0.07	0.9339
Type	2	111.54	**0.0001**
Depth*Type	4	1.24	0.3622
Residual	9		
Site C	**DF**	**F**	**p**
Depth	2	0.13	0.8809
Type	2	1149.34	**0.0001**
Depth*Type	4	1.28	0.3474
Residual	9		

2-way ANOVA for effects of sediment depth (0-2, 2-5, 5-10 cm) and time (June-October) on organic content of cores collected from Nanjemoy Creek. Numbers in bold are significant; n = 3 for depth and n = 5 for time.

Site A	**DF**	**F**	**p**
Depth	2	1.35	0.2749
Time	4	23.76	**0.0001**
Depth*Time	8	0.43	0.8942
Residual	30		
Site B	**DF**	**F**	**p**
Depth	2	0.73	0.4914
Time	4	2.45	0.6740
Depth*Time	8	0.37	0.9268
Residual	30		
Site C	**DF**	**F**	**p**
Depth	2	2.22	0.1265
Time	4	0.65	0.6312
Depth*Time	8	0.42	0.8971
Residual	29		

APPENDIX 6

Average sediment porewater nutrient concentrations for each site in Nanjemoy Creek from June to October. Concentrations are reported as the mean ± SE.

	Site A	Site B	Site C
NO_2^- + NO_3^- µM			
June			
2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
July			
2	1.11 ± 0.34	4.30 ± 2.88	0.50 ± 0.09
5	1.37 ± 0.72	0.00 ± 0.00	0.00 ± 0.00
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
August			
2	26.68 ± 12.10	16.93 ± 2.15	0.13 ± 0.13
5	5.53 ± 5.53	2.82 ± 2.82	0.00 ± 0.00
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
September			
2	31.35 ± 1.52	34.34 ± 3.82	2.28 ± 1.22
5	11.71 ± 11.42	13.04 ± 11.37	0.00 ± 0.00
10	0.00 ± 0.00	1.33 ± 1.19	0.00 ± 0.00
October			
2	6.52 ± 0.71	9.92 ± 4.34	4.38 ± 1.77
5	3.79 ± 1.93	7.84 ± 4.12	2.64 ± 0.65
10	0.00 ± 0.00	1.96 ± 0.99	0.00 ± 0.00

APPENDIX 6 continued

	Site A	Site B	Site C
NH_4^+ µM			
June			
2	110.46 ± 10.56	28.52 ± 4.85	63.39 ± 34.19
5	169.88 ± 39.32	37.15 ± 6.18	76.19 ± 38.75
10	129.18 ±19.94	27.60 ± 6.83	65.62 ± 35.25
July			
2	103.47 ± 18.62	57.73 ± 7.21	18.03 ± 8.01
5	198.59 ± 27.34	86.34 ± 16.74	34.00 ± 10.74
10	306.74 ± 44.44	64.84 ± 18.78	21.17 ± 1.53
August			
2	130.68 ± 35.26	56.53 ± 19.52	15.97 ± 1.19
5	250.66 ± 72.38	61.04 ± 36.31	21.61 ± 5.50
10	259.18 ± 44.39	21.50 ± 7.30	30.40 ± 13.21
September			
2	112.95 ± 19.61	63.23 ± 8.00	17.07 ± 3.62
5	162.80 ± 20.71	56.89 ± 22.32	23.73 ± 0.37
10	140.66 ± 26.48	49.50 ± 15.92	18.05 ± 9.33
October			
2	43.21 ± 4.90	48.99 ± 15.59	57.81 ± 14.23
5	85.29 ± 25.35	48.83 ± 13.69	79.71 ± 31.73
10	108.10 ± 18.84	47.18 ± 8.37	50.48 ± 4.10

APPENDIX 6 continued

	Site A	Site B	Site C
PO_4^{-3} µM			
June			
2	0.71 ± 0.17	0.06 ± 0.06	0.60 ± 0.16
5	0.21 ± 0.13	0.25 ± 0.03	0.44 ± 0.15
10	0.17 ± 0.02	0.33 ± 0.06	0.27 ± 0.09
July			
2	0.74 ± 0.26	0.38 ± 0.09	0.48 ± 0.25
5	0.69 ± 0.45	0.16 ± 0.06	0.49 ± 0.14
10	0.43 ± 0.26	0.24 ± 0.08	0.13 ± 0.03
August			
2	0.74 ± 0.34	0.59 ± 0.24	0.48 ± 0.06
5	0.58 ± 0.28	0.33 ± 0.14	0.82 ± 0.48
10	0.62 ± 0.34	0.23 ± 0.05	0.63 ± 0.25
September			
2	0.59 ± 0.15	0.40 ± 0.04	0.47 ± 0.05
5	0.29 ± 0.03	0.45 ± 0.11	1.12 ± 0.78
10	0.28 ± 0.02	0.48 ± 0.16	0.20 ± 0.10
October			
2	0.75 ± 0.09	0.50 ± 0.08	0.58 ± 0.08
5	0.34 ± 0.05	0.22 ± 0.03	0.29 ± 0.06
10	0.34 ± 0.01	0.22 ± 0.03	0.15 ± 0.07

APPENDIX 7

2-way ANOVA for effects of time (June-October) and depth (0-2, 2-5, 5-10 cm) on sediment porewater nutrient concentrations from all sampling sites in Nanjemoy Creek. Numbers in bold are significant; n = 5 for date, n = 3 for depth.

$NO_2^- + NO_3^-$ µM			
Site A	**DF**	**F**	**P**
Date	4	5.79	**0.0014**
Depth	2	10.65	**0.0003**
Date*Depth	8	2.84	**0.0179**
Residual	30		
Site B			
Date	4	3.50	**0.0186**
Depth	2	11.20	**0.0002**
Date*Depth	8	1.60	0.1663
Residual	30		
Site C			
Date	4	8.66	**<0.0001**
Depth	2	8.10	**0.0015**
Date*Depth	8	2.94	**0.0151**
Residual	30		

NH_4^+ µM			
Site A	**DF**	**F**	**p**
Date	4	8.48	**0.0001**
Depth	2	10.47	**0.0004**
Date*Depth	8	1.62	0.1624
Residual	30		
Site B			
Date	4	2.34	0.0778
Depth	2	1.24	0.3040
Date*Depth	8	0.44	0.8899
Residual	30		
Site C			
Date	4	4.57	**0.0053**
Depth	2	0.59	0.5618
Date*Depth	8	0.13	0.9973
Residual	30		

PO_4^{-3} µM			
Site A	**DF**	**F**	**p**
Date	4	0.899	0.4784
Depth	2	3.424	**0.0473**
Date*Depth	8	0.227	0.9827
Residual	26		
Site B			
Date	4	1.61	0.2005
Depth	2	1.71	0.1995
Date*Depth	8	1.03	0.4394
Residual	28		
Site C			
Date	4	0.59	0.6742
Depth	2	1.75	0.1934
Date*Depth	8	0.71	0.6796
Residual	27		

APPENDIX 8

Mean above ground biomass of *V. americana* shoots collected from Nanjemoy Creek. Numbers are g dry weight m^{-2} ± SE.

A

Date	Site A	Site B	Site C
April	11.28 ± 9.78	1.94 ± 0.55	0.31 ± 0.06
May	50.67 ± 8.34	2.55 ± 0.27	5.90 ± 1.10
June	102.32 ± 7.69	.	.
July	81.97 ± 11.99	122.01 ± 8.27	75.95 ± 9.78
August	102.20 ± 20.27	99.97 ± 9.74	84.65 ± 7.52
September	140.19 ± 25.86	146.34 ± 35.25	339.45 ± 210.23
October	99.39 ± 16.03	27.30 ± 18.31	84.15 ± 24.80

Mean below ground biomass of *V. americana* shoots collected from Nanjemoy Creek. Numbers are g dry weight m^{-2} ± SE.

Date	Site A	Site B	Site C
April	28.51 ± 7.10	9.52 ± 1.83	1.05 ± 0.58
May	7.81 ± 1.70	53.77 ± 7.78	10.13 ± 4.44
June	43.60 ± 1.00	.	.
July	51.25 ± 15.08	54.97 ± 13.33	47.22 ± 13.26
August	52.07 ± 2.32	60.50 ± 13.11	32.79 ± 6.25
September	147.67 ± 98.92	84.80 ± 21.33	183.16 ± 69.97
October	66.03 ± 4.14	33.29 ± 7.60	42.44 ± 2.36

APPENDIX 9

Temperature (°C), salinity (psu) and DO (mg l^{-1}) of the water column in the temperature experiment. No data was collected on days 1 and 2.

Day	Treatment	Temp °C	Salinity psu	DO mg l^{-1}
3	13	8.7	0.01	12.23
4	13	10.3	0.01	11.81
5	13	10.2	0.01	12.11
6	13	11.1	0.02	11.68
7	13	9.1	0.01	12.00
8	13	13.5	0.02	10.74
9	13	13.9	0.01	10.54
10	13	16.9	0.01	9.78
11	13	14.8	0.01	9.61
12	13	13.5	0.02	7.68
13	13	15.6	0.01	10.21
14	13	15.5	0.02	10.27
15	13	13.1	0.01	10.05
16	13	13.4	0.02	10.92
17	13	15.6	0.01	10.16
18	13	15.5	0.02	10.42
19	13	13.9	0.01	10.08
20	13	13.7	0.02	11.00
21	13	8.9	0.01	12.25
3	22	21.6	0.01	8.72
4	22	22.6	0.01	8.30
5	22	26.1	0.02	7.75
6	22	18.3	0.02	8.86
7	22	22.3	0.02	8.48
8	22	24.0	0.02	8.09
9	22	16.5	0.02	9.77
10	22	19.7	0.02	8.87
11	22	22.7	0.02	8.36
12	22	24.9	0.02	7.86
13	22	24.2	0.02	8.09
14	22	20.1	0.01	8.60
15	22	23.5	0.02	8.32
16	22	25.4	0.02	7.87
17	22	21.9	0.02	8.71
18	22	17.1	0.02	8.79

Day	Treatment	Temp °C	Salinity psu	DO mg l^{-1}
19	22	25.0	0.01	7.97
20	22	23.5	0.02	8.20
21	22	22.2	0.02	8.69
3	25	21.0	0.01	8.26
4	25	24.5	0.01	8.02
5	25	20.2	0.01	8.05
6	25	27.6	0.02	7.01
7	25	22.0	0.01	8.31
8	25	25.6	0.02	7.76
9	25	24.6	0.01	6.13
10	25	24.3	0.02	7.77
11	25	22.7	0.01	8.01
12	25	25.9	0.02	7.68
13	25	24.4	0.01	6.42
14	25	25.2	0.02	7.80
15	25	23.7	0.01	7.91
16	25	26.7	0.02	7.58
17	25	26.8	0.01	6.52
18	25	24.9	0.02	7.82
19	25	23.2	0.01	8.02
20	25	26.0	0.02	7.78
21	25	25.5	0.02	6.56
3	29	27.5	0.01	7.42
4	29	26.4	0.01	7.63
5	29	24.1	0.01	8.03
6	29	29.9	0.01	7.05
7	29	28.4	0.01	7.11
8	29	27.4	0.01	6.46
9	29	25.1	0.01	6.84
10	29	32.7	0.01	6.49
11	29	29.4	0.02	6.99
12	29	28.7	0.01	7.09

APPENDIX 9 continued

Day	Treatment	Temp °C	Salinity psu	DO mg l^{-1}
13	29	25.5	0.01	6.99
14	29	32.4	0.01	6.63
15	29	30.4	0.02	6.79
16	29	24.0	0.01	7.00
17	29	30.4	0.02	6.57
18	29	33.3	0.02	6.44
19	29	30.7	0.02	6.63
20	29	23.9	0.01	6.39
21	29	32.9	0.02	6.37

APPENDIX 10

Temperature (°C), salinity (psu), and DO (mg l^{-1}) water column concentrations in the salinity experiment. Data reported as mean ± SE.

Day	Treatment	Temperature ° C	Salinity psu	DO mg l^{-1}
1	0	24.7 ± 0.2	0.14 ± 0.16	8.16 ± 0.02
1	5	24.7 ± 0.1	4.98 ± 0.04	7.98 ± 0.04
1	10	24.8 ± 0.1	10.05 ± 0.13	7.94 ± 0.02
1	15	24.7 ± 0.2	14.83 ± 0.06	7.88 ± 0.08
2	0	24.9 ± 0.2	0.10 ± 0.04	8.10 ± 0.04
2	5	24.9 ± 0.2	4.98 ± 0.04	8.01 ± 0.04
2	10	25.0 ± 0.2	10.16 ± 0.09	8.35 ± 0.33
2	15	25.0 ± 0.2	14.91 ± 0.08	8.88 ± 0.61
3	0	25.1 ± 0.2	0.12 ± 0.04	8.05 ± 0.09
3	5	25.2 ± 0.2	4.96 ± 0.09	7.90 ± 0.04
3	10	25.3 ± 0.2	10.11 ± 0.09	7.77 ± 0.17
3	15	25.3 ± 0.2	14.88 ± 0.09	7.38 ± 0.05
4	0	24.7 ± 0.1	0.11 ± 0.03	8.03 ± 0.05
4	5	24.7 ± 0.1	5.15 ± 0.03	7.92 ± 0.05
4	10	24.7 ± 0.1	10.18 ± 0.05	7.65 ± 0.04
4	15	24.7 ± 0.2	15.08 ± 0.09	7.41 ± 0.08
5	0	24.4 ± 0.2	0.10 ± 0.04	8.03 ± 0.03
5	5	24.4 ± 0.2	5.08 ± 0.08	7.87 ± 0.04
5	10	24.4 ± 0.2	10.30 ± 0.11	7.62 ± 0.05
5	15	24.4 ± 0.2	15.10 ± 0.11	7.37 ± 0.07
6	0	24.5 ± 0.2	0.11 ± 0.04	8.08 ± 0.02
6	5	24.5 ± 0.2	5.08 ± 0.07	7.91 ± 0.04
6	10	24.5 ± 0.2	10.34 ± 0.12	7.65 ± 0.04
6	15	24.5 ± 0.2	15.25 ± 0.14	7.34 ± 0.05
18	0	24.4 ± 0.1	0.13 ± 0.04	8.12 ± 0.02
18	5	24.5 ± 0.1	5.47 ± 0.10	7.87 ± 0.02
18	10	24.5 ± 0.1	11.17 ± 0.07	7.64 ± 0.04
18	15	24.5 ± 0.1	16.47 ± 0.31	7.52 ± 0.06
33	0	24.4 ± 0.1	0.23 ± 0.06	8.14 ± 0.07
33	5	24.5 ± 0.1	6.19 ± 0.40	7.94 ± 0.10
33	10	24.5 ± 0.1	11.65 ± 0.08	7.69 ± 0.06
33	15	24.5 ± 0.1	17.43 ± 0.43	7.43 ± 0.06

APPENDIX 11

Temperature (°C), salinity (psu), and DO (mg l^{-1}) water column concentrations of the STBD experiment. Data are reported as mean ± SE.

Day	Tank	Temperature °C	Salinity psu	DO mg l^{-1}
1	1	25.0 ± 0.0	0.18 ± 0.00	8.43 ± 0.05
1	2	23.7 ± 1.0	0.19 ± 0.00	8.28 ± 0.01
1	3	24.3 ± 0.0	0.19 ± 0.00	8.31 ± 0.01
2	1	25.0 ± 0.0	0.19 ± 0.00	8.31 ± 0.06
2	2	24.7± 0.0	0.19 ± 0.00	8.26 ± 0.01
2	3	24.4 ± 0.0	0.19 ± 0.00	8.31 ± 0.00
3	1	25.0 ± 0.0	0.19 ± 0.00	8.85 ± 0.08
3	2	24.8 ± 0.0	0.19 ± 0.00	8.83 ± 0.01
3	3	24.5 ± 0.0	0.20 ± 0.00	8.95 ± 0.04
4	1	25.0 ± 0.1	0.19 ± 0.00	8.40 ± 0.01
4	2	24.8 ± 0.0	0.19 ± 0.00	8.46 ± 0.01
4	3	24.5 ± 0.0	0.20 ± 0.00	8.55 ± 0.01
5	1	25.1 ± 0.0	0.19 ± 0.00	8.60 ± 0.04
5	2	24.8 ± 0.0	0.19 ± 0.00	8.55 ± 0.01
5	3	24.5 ± 0.0	0.20 ± 0.00	8.57 ± 0.00
6	1	25.0 ± 0.0	0.19 ± 0.00	8.34 ± 0.00
6	2	24.7 ± 0.0	0.19 ± 0.00	8.38 ± 0.01
6	3	24.4 ± 0.0	0.20 ± 0.00	8.42 ± 0.01
7	1	25.1 ± 0.0	0.19 ± 0.00	8.26 ± 0.03
7	2	24.7 ± 0.0	0.19 ± 0.00	8.27 ± 0.00
7	3	24.5 ± 0.0	0.20 ± 0.00	8.29 ± 0.01
8	1	25.1 ± 0.0	0.19 ± 0.00	8.04 ± 0.05
8	2	24.8 ± 0.0	0.19 ± 0.00	7.99 ± 0.02
8	3	24.5 ± 0.0	0.20 ± 0.00	7.99 ± 0.00
9	1	25.0 ± 0.0	0.19 ± 0.00	8.15 ± 0.03
9	2	24.7 ± 0.0	0.19 ± 0.00	8.12 ± 0.03
9	3	24.4 ± 0.0	0.20 ± 0.00	8.07 ± 0.02
10	1	24.9 ± 0.0	0.19 ± 0.00	8.12 ± 0.03
10	2	24.7 ± 0.0	0.19 ± 0.00	8.12 ± 0.00
10	3	24.4 ± 0.0	0.20 ± 0.00	8.12 ± 0.01
11	1	24.9 ± 0.0	0.20 ± 0.00	8.16 ± 0.04
11	2	24.6 ± 0.0	0.20 ± 0.00	8.22 ± 0.00
11	3	24.3 ± 0.0	0.21 ± 0.00	8.26 ± 0.01
12	1	24.9 ± 0.0	0.20 ± 0.00	8.19 ± 0.01
12	2	24.7 ± 0.0	0.20 ± 0.00	8.16 ± 0.01

Day	Tank	Temperature °C	Salinity psu	DO mg l^{-1}
12	3	24.4 ± 0.0	0.21 ± 0.00	8.19 ± 0.01
15	1	24.9 ± 0.0	0.20 ± 0.00	8.13 ± 0.01
15	2	24.7 ± 0.0	0.20 ± 0.00	8.14 ± 0.01
15	3	24.4 ± 0.0	0.21 ± 0.00	8.21 ± 0.01
21	1	25.0 ± 0.0	0.21 ± 0.00	8.30 ± 0.04
21	2	24.6 ± 0.0	0.21 ± 0.00	8.29 ± 0.01
21	3	24.4 ± 0.0	0.22 ± 0.00	8.32 ± 0.01
23	1	24.9 ± 0.0	0.21 ± 0.00	8.43 ± 0.06
23	2	24.7 ± 0.0	0.21 ± 0.00	8.35 ± 0.01
23	3	24.4 ± 0.0	0.22 ± 0.00	8.39 ± 0.01
28	1	25.0 ± 0.0	0.21 ± 0.00	8.17 ± 0.01
28	2	24.7 ± 0.0	0.21 ± 0.00	8.18 ± 0.01
28	3	24.4 ± 0.0	0.22 ± 0.00	8.17 ± 0.01

APPENDIX 12

Mean water column nutrient concentrations ± SE before (09/02/04) and after (09/19/04) the salinity experiment.

Date	Treatment	$NO_2^-+NO_3^-$ µM	NH_4^+ µM	PO_4^{-3} µM
09/02/04	0	0.33 ± 0.16	0.89 ± 0.25	0.20 ± 0.02
09/02/04	5	4.79 ± 0.08	0.56 ± 0.24	0.45 ± 0.01
09/02/04	10	8.29 ± 0.15	0.48 ± 0.05	0.46 ± 0.02
09/02/04	15	12.29 ± 0.20	0.46 ± 0.07	0.86 ± 0.04
09/19/04	0	0.97 ± 0.39	1.67 ± 1.03	0.07± 0.02
09/19/04	5	0.31 ± 0.08	1.17 ± 0.34	0.07 ± 0.02
09/19/04	10	0.19 ± 0.01	0.99 ± 0.09	0.03 ± 0.01
09/19/04	15	1.03 ± 0.92	2.10 ± 1.63	0.01 ± 0.01

2-way ANOVA on effects of time and salinity on water column nutrient concentrations before (09/02/02) and after (09/19/04) the salinity experiment. Numbers in bold are significant and n=2 for date, n=4 for salinity.

	DF	F	p
$NO_2^-+NO_3^-$ µM			
Date	1	491.24	**<0.0001**
Salinity	3	95.14	**<0.0001**
Date*Salinity	3	95.40	**<0.0001**
Residual	24		
NH_4^+ µM			
Date	1	3.18	0.0871
Salinity	3	0.32	0.8116
Date*Salinity	3	0.27	0.8454
Residual	24		
PO_4^{-3} µM			
Date	1	940.59	**<0.0001**
Salinity	3	70.55	**<0.0001**
Date*Salinity	3	100.97	**<0.0001**
Residual	24		

APPENDIX 13

Mean water column nutrient concentrations ± SE for temperature experiment before (06/10/04) and after (06/24/04) the temperature experiment.

Date	Temperature °C	$NO_2^-+NO_3^-$ µM	NH_4^+ µM	PO_4^{-3} µM
06/10/04	13	0.38 ± 0.06	7.71 ± 3.62	6.20 ± 1.33
06/10/04	22	0.28 ± 0.05	9.58 ± 4.82	5.12 ±1.31
06/10/04	25	0.29 ± 0.09	14.01 ± 11.95	6.27 ± 0.54
06/10/04	29	0.26 ± 0.04	4.01 ± 1.59	5.08 ± 0.72
06/24/04	13	0.46 ± 0.08	15.49 ± 4.20	6.77 ± 1.38
06/24/04	22	0.35 ± 0.06	24.99 ± 9.55	5.14 ±1.57
06/24/04	25	1.00 ± 0.68	31.12 ± 10.13	8.73 ± 1.18
06/24/04	29	0.33 ±0.05	39.08 ±10.46	7.45 ± 0.48

2-way ANOVA on effects of time and temperature on water column nutrient concentrations during temperature experiment. Numbers in bold are significant; n = 2 for date, n = 4 for temperature.

	DF	F	p
$NO_2^-+NO_3^-$ µM			
Date	1	1.74	0.1997
Temp	3	0.82	0.4971
Date*Temp	3	0.81	0.5004
Residual	24		
NH_4^+ µM			
Date	1	11.30	**0.0026**
Temp	3	0.79	0.5102
Date*Temp	3	1.06	0.3831
Residual	24		
PO_4^{-3} µM			
Date	1	2.86	0.1038
Temp	3	1.48	0.2461
Date*Temp	3	0.60	0.6189
Residual	24		

APPENDIX 14

Mean water column nutrients ± SE for sediment type burial depth experiment. ID is the tank number, T1 = tank 1, T2 = tank 2, T3 = tank 3.

ID	$NO_2^-+NO_3^-$ µM	NH_4^+ µM	PO_4^{-3} µM
T1	1.07 ± 0.02	0.92 ± 0.08	17.35 ± 0.38
T2	1.12 ± 0.02	0.92 ± 0.24	17.46 ± 0.03
T3	0.12 ± 0.01	0.32 ± 0.03	17.34 ± 0.03

1-way ANOVA of tank effects on water column nutrients. Numbers in bold are significant and n = 3 for tanks.

	DF	F	p
$NO_2^-+NO_3^-$ µM			
Tank	2	3362.15	**<0.0001**
Residual	6		
NH_4^+ µM			
Tank	2	16.92	**0.0034**
Residual	6		
PO_4^{-3} µM			
Tank	2	0.27	0.7749
Residual	6		

APPENDIX 15

Mean sediment porewater nutrient concentrations ± SE for sediment type burial depth experiment.

% NS	$NO_2^-+NO_3^-$ µM	NH_4^+ µM	PO_4^{-3} µM
100 %	0.00 ± 0.00	269.46 ± 90.10	2.97 ± 0.70
75 %	0.00 ± 0.00	379.77 ± 125.53	0.55 ± 0.02
50 %	0.00 ± 0.00	370.76 ± 20.91	0.39 ± 0.02
25 %	sample lost	sample lost	sample lost
0 %	0.00 ± 0.00	405.57 ± 23.98	0.51 ± 0.06

1-way ANOVA on effects of sediment type on porewater nutrient concentrations during sediment type burial depth experiment. Numbers in bold are significant; n = 4 for sediment type.

STBD	DF	F	p
$NO_2^-+NO_3^-$ µM			
% NS	3	1.04	0.4277
Residual	8		
NH_4^+ µM			
% NS	3	0.51	0.691
Residual	7		
PO_4^{-3} µM			
% NS	3	8.94	**0.0124**
Residual	6		

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VITA

JESSIE J. CAMPBELL

Jessie was born in Easton, Maryland on July 9, 1981. After graduating from Easton High School in 1999 she went on to earn a B.S. in Biology from Chowan College in 2003. Jessie began her masters program in the College of William and Mary, School of Marine Science in 2003.